Interdependence between Plasma Ionized Calcium and Hemodynamic Performance

L. J. Drop

INTERDEPENDENCE BETWEEN PLASMA IONIZED CALCIUM AND HEMODYNAMIC PERFORMANCE

From the Anesthesia Laboratory of the Harvard Medical School at Massachusetts General Hospital, Boston, Massachusetts 02114.
Supported in part by NIGMS Grant GM 15904-05

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PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN
DOCTOR IN DE GENEESKUNDE
AAN DE KATHOLIEKE UNIVERSITEIT TE NIJMEGEN
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF. MR. F. J. F. M. DUYNSTEE
VOLGENS BESLUIT VAN HET COLLEGE VAN DECANEN
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LAMBERTUS JOHANNES DROP

GEBOREN TE

VLAARDINGEN

MASSACHUSETTS GENERAL HOSPITAL PRINTING OFFICE
BOSTON, MASSACHUSETTS

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Dedicated to

Dr. M. B. Laver

PREFACE

Completion of this thesis gives me an opportunity to express my sincere feelings of gratitude to my parents, who have shown warm interest and provided support during my years in medical school, during my years of gaining experience as a Famulus in Klagenfurt and Glarus as well as during my years of postgraduate education in Yverdon, Switzerland; Morgantown, West Virginia and Boston, Massachusetts.

Dank gilt Herrn Prim. Dr. K. Rauchenwald, Vorstand der Abteilung für Urologie am Landeskrankenhaus in Klagenfurt. Die praktische Erfahrung dieser Famulatur war sehr nützlich in den späteren klinischen Semestern. Dank auch an Herrn Dr. H. R. Bloch, Chefarzt am Kantonsspital in Glarus. Ich bewahre die besten Erinnerungen an die Unterassistentenzeit in Glarus.

Je témoigne ma reconnaissance à Monsieur le Docteur J.-L. Wyss, médecin-chef du service de médecine de l'Hôpital d'Yverdon. Ce stage en médecine représente un époque tout à fait unique de ma vie.

Thanks are due to Dr. B. Zimmermann, Professor and Chairman, Department of Surgery, West Virginia University Medical Center, Morgantown. Indebtedness is expressed to Dr. W. H. Moran, Professor of Surgery and Biophysics, West Virginia University Medical Center, Morgantown. His experience and guidance in the management of fluids and electrolytes in the acutely burned patient was very valuable.

A word of appreciation is directed to Dr. Henry K. Beecher, Henry Isaiah Dorr Professor of Research in Anaesthesia, Emeritus, Harvard Medical School, Dr. Richard J. Kitz, Henry Isaiah Dorr Professor of Anaesthesia, Harvard Medical School and the staff of the Anesthesia Department at Massachusetts General Hospital. The three year program in this excellent and research oriented department provided an immeasurably valuable experience.

The support, cooperation and interest of the following persons is gratefully acknowledged: Dr. Edward Lowenstein, Associate Professor of Anaesthesia, Harvard Medical School for his help in obtaining blood samples on various occasions during the study; Dr. Neville J. Davis, Clinical Fellow in Anaesthesia, Massachusetts General Hospital for allowing us to study patients subjected to deliberate hypotensive anesthesia; Dr. David J. Cullen, Assistant Professor of Anaesthesia, Harvard Medical School for allowing us to follow patients in the Recovery

Rooms; Dr. Henning Pontoppidan, Associate Professor of Anaesthesia, Harvard Medical School for allowing us to follow patients in the Respiratory Unit; Dr. Porter H. Smith and Dr. Thomas J. Keenan from the Massachusetts Eye and Ear Infirmary, the staff of the Anesthesia Research Laboratory and of the Acute Care Laboratory for their technical and analytical support; and Mr. M. B. Scherperel for unexcelled help with the manuscript.

To all not explicitly mentioned who have been of help in the completion of this study, I take this opportunity to express my sincere gratitude.

LEGEND

The following symbols and abbreviations will be used:

Ca^{++}	*in text* represents ionized calcium without specification of medium (aqueous solutions, serum or plasma)
Ca^{++}	*in figures* represents Ca^{++}_P except when noted otherwise
Ca^{++}_S	Ionized calcium concentration in serum (mM/L)
Ca^{++}_P	Ionized calcium concentration in plasma (mM/L)
Ca^{++}_{WB}	Ionized calcium concentration in whole blood (mM/L)
[Ca]	Total calcium concentration (mM/L)
P_I	Inorganic phosphate concentration (mM/L)
T.P.	Total protein concentration (g/100 ml)
[Mg]	Total magnesium concentration (mEq/L)
C.I.	Cardiac index ($L/min/M^2$)
S.I.	Stroke index ($ml/min/M^2$)
B.P.	Arterial blood pressure (torr)
C.V.P.	Central venous pressure (torr)

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"On ne peut présenter la science si on ne la repense pas chaque fois à nouveau"

W. von Humboldt

CHAPTER I

INTRODUCTION

A. CLINICAL APPLICATION OF IONIZED CALCIUM MEASUREMENTS

The important functions of the calcium ion in many physiological processes have long been recognized. Examples of processes in which it plays a regulatory role include neuromuscular transmission, coagulation of blood, coupling of muscle excitation and contraction, the sequence of muscle activation and relaxation, and the release of insulin.[76]

Almost a century ago, Ringer[120] made the observation that muscle contraction in an isolated perfused heart preparation occurs only if calcium ions are present in the bath fluid. The dependence of myocardial contraction on extracellular calcium has been subsequently demonstrated by McLean and Hastings[81-83] in their classical frog heart experiments from which they derived a quantitative bio-assay.

In spite of recognition of the importance of Ca^{++} for adequate hemodynamic performance, there is a lack of data on their interdependence in man.

It has been generally assumed that in the absence of overt alterations in endocrine function, acute changes in Ca^{++} appear only in neonates and infants or following intravenous infusion of citrate or phosphate. Moreover, it has been assumed that calcium can be readily mobilized whenever blood levels are diminished. However, data substantiating this hypothesis are lacking.

Acute changes in oxygenation, acid-base balance and electrolyte levels are commonplace in critically ill patients. Acute alterations in plasma calcium levels in such patients have not been identified in the past. Recognition of the changes in Ca^{++} is now possible since a practical method of rapid and repeated Ca^{++} measurement has become available. It is widely recognized that successful management of these various abnormalities is largely dependent upon appropriate interpretation of repeated laboratory measurements.[67]

The purpose of this study was to validate the use of the calcium selective electrode for clinical purposes and to describe the interdependence between hemodynamic function and Ca^{++}_i in patients with various underlying disorders.

B. REVIEW OF METHODOLOGY

Calcium is present in plasma in two different physicochemical fractions as described in 1911 by Rona and Takahashi[124]: *bound* to proteins and *complexed* to anions; and in the *ionized* form (fig. 1). The classical frog heart experiments of McLean and Hastings[81-83] have demonstrated that the unbound, ionized moiety is the physiologically active one. These authors developed a nomogram (fig. 2) from which Ca^{++}_P values can be predicted on the basis of alterations in [Ca] and total protein (T.P.) concentration.

Attempts made to quantitate the ionized moiety both in aqueous solutions and in biological media have met with limited success.

The colorimetric assay for Ca^{++} using murexide (ammonium purpurate) is applicable to ultrafiltrates[31, 125, 140] and serum[79] but requires a considerable volume of blood and is time consuming. It is not suitable for rapid and repeated Ca^{++} measurements in the clinical laboratory. Ultrafiltration itself is known to evoke changes in Ca^{++} concentration.[84, 90] This is particularly important when comparing "normal" values reported by different authors (table 3).

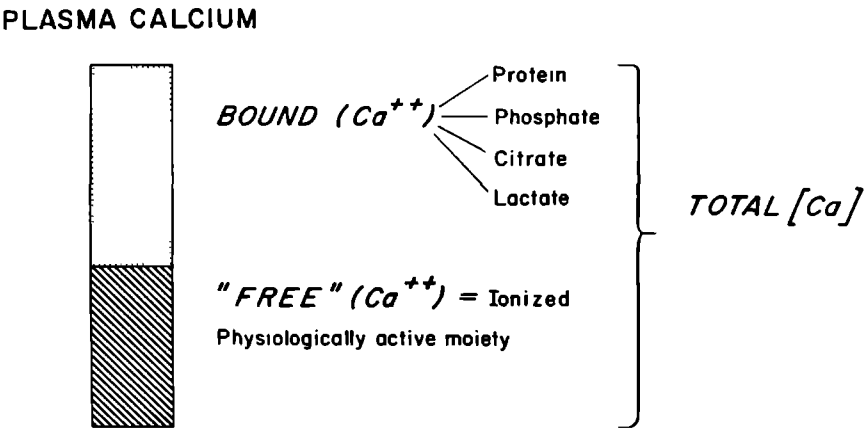

Fig. 1 — The physicochemical fractions of plasma calcium.

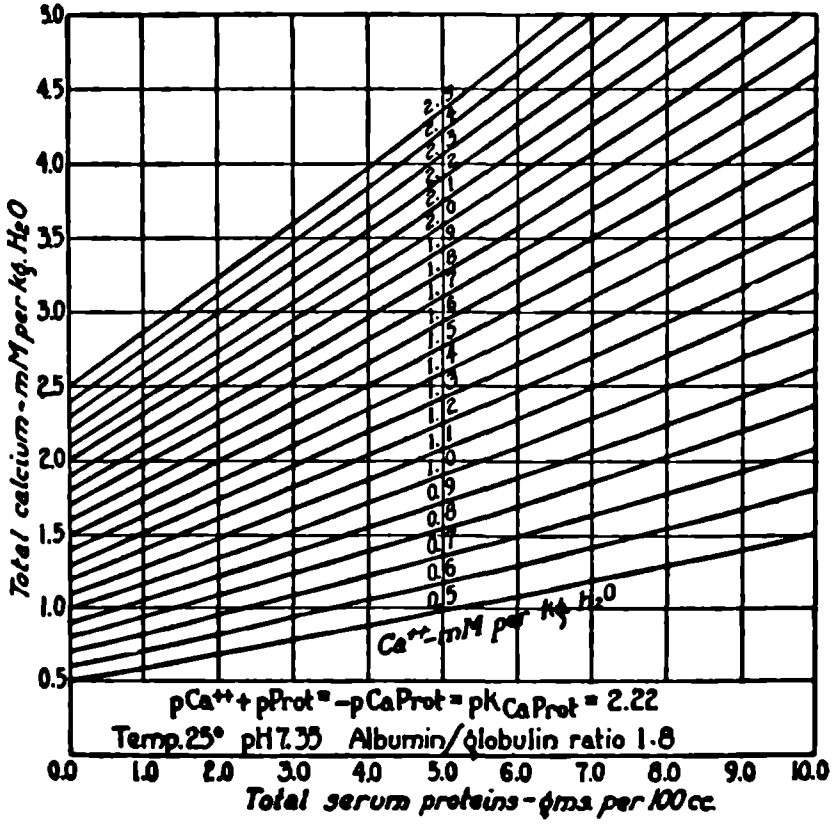

Fig. 2 — The McLean-Hastings nomogram. (From McLean, F. C., and Hastings, A. B.: The State of Calcium in the Fluids of the Body. J. Biol. Chem. 108:285,1935) Reprinted with permission.

Potentiometric measurement of Ca^{++} activity has been described by Fosbinder,[34] who developed a calcium amalgam electrode; Tendeloo,[148, 149] who utilized a paraffin membrane electrode; and Abrams,[2] who presented data using a collodion membrane electrode. In each of these techniques, the major drawbacks were inadequate sensitivity and lack of reproducibility in solutions containing proteins. Generally, glass electrodes have been found to be too insensitive either for research purposes or in the clinical laboratory.[85]

A simple, reproducible potentiometric technique became available with the development of a calcium-selective electrode and is suitable for analyses of biological fluids.[85, 90, 127]

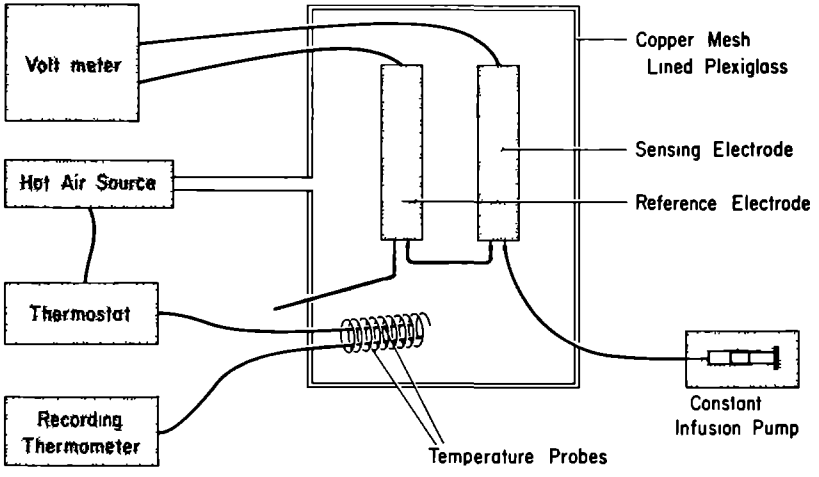

Fig. 3 — Diagram of the electrode system. The flow-through sensing and reference electrodes were placed in a plexiglass box, lined with copper mesh to protect from electrical noise, and maintained at 37°C. The plasma samples were delivered to the electrode by a constant infusion pump. Electrode potentials were read from an electrometer.

C. PRINCIPLE OF THE POTENTIOMETRIC DETERMINATION OF IONIZED CALCIUM

The system* used in this study (fig. 3) consists of a flow-through calcium sensing electrode (model 99-20) and a calomel reference electrode (model 99-20). A constant infusion pump (model 88-20) delivers the sample to the electrode system at a rate of 0.05 ml per minute. Electrode potentials are read from an electrometer (model 801). The sensing electrode is shown in cross section in figure 4.

The impermeable electrode housing separates two cylindrical outer chambers from a centrally placed cylindrical inner chamber. The outer chambers are continuous at the bottom and contain the liquid ion-exchange resin, a solution of calcium didecylphosphate in di-*n*-octylphenyl phosphonate. A thin film of this ion-exchange resin is formed around a porous cellulose membrane separating the inner chamber from the sample. The inner chamber contains 0.1 M $CaCl_2$ solution ("internal filling solution") and a Ag-AgCl internal reference electrode. The 0.1 M $CaCl_2$ solution in contact with the inner surface of the membrane provides a stable calcium potential between the inner

* Orion Research Inc., Cambridge, Massachusetts

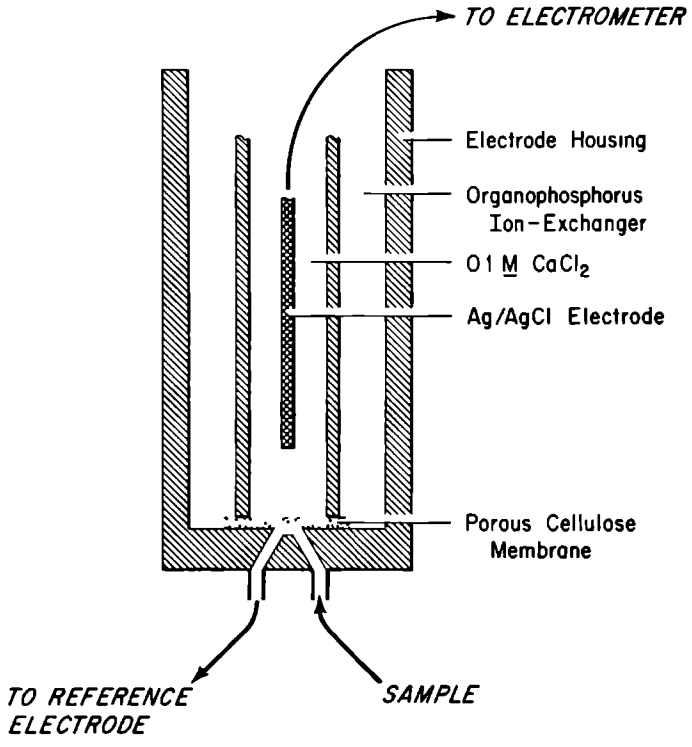

Fig. 4 — Schematic longitudinal cross section of the calcium selective electrode The outer cylindrical chambers are filled with the organophosphorus ion-exchanger, which forms a thin film around the porous membrane The inner chamber is filled with 0 1 M $CaCl_2$. The internal reference (Ag/AgCl) electrode is connected to the electrometer.

surface of the membrane and the organophosphorus ion-exchanger; the chloride ion provides a stable potential between the reference electrode (Ag-AgCl) and the filling solution. Thus, the sample is in contact with the outer membrane surface and changes in electrode potential may be assumed to be due to differences in calcium activity of the sample. If the organophosphorus ion-exchanger is indeed highly selective for calcium then only calcium ions in the sample can exchange with the organic calcium salt. The ideal equilibrium potential across the membrane is given by the Nernst equation:

$$E = E_o + \frac{RT}{nF} \log A_{Ca^{++}}$$

where E = potential developed by the electrode system (all junctions); E_o the potential developed by the reference electrode and internal solutions; $A_{Ca^{++}}$ the ionic activity in the sample; R the gas constant; T the absolute temperature; n the charge on the ion and F the Faraday constant.

Close correlation between electrode behavior and theoretical values predicted by the Nernst equation is indicative of ion selectivity. In other words an ideal membrane will result in an electrode potential proportional to the logarithm of the ion activity in the sample. Data from the literature[106, 116] have indicated that in the range of 0.1 m$\underline{M}$ to 1 $\underline{M}$ the electrode conforms to the behavior predicted by the Nernst equation.

Theoretical considerations concerning the properties of ions in solution can be derived from ideal gas laws on the assumption that ions in very dilute solutions behave like gas molecules. This parallel, however, does not apply to more concentrated solutions, primarily because interionic electrostatic interactions limit free movement. Determinations of conductivity, vapor pressure and freezing point depression have confirmed the concept that the apparent number of free ions (*i.e., activity*) in solution is less than calculated from molar concentration assuming complete dissociation of salts. The apparent number of independent ions has been referred to as the *ionic activity.*[72] Activity is related to concentration by the following equation:

$$A = \gamma C$$

where A = activity, γ = activity coefficient and C = molar concentration of the ionic species under consideration. At infinite dilution, γ equals 1. The activity coefficient is affected primarily by temperature and interionic electrostatic interactions (*i.e., ionic strength*). To account for non-ideal behavior of solutions caused by these electrostatic interactions, Lewis[72] has developed the concept of *ionic strength,* related to the concentrations and valencies of all ions present in solution and defined by the following equation:

$$\mu = \tfrac{1}{2}[C_aZ_a^2 + C_bZ_b^2 + \ldots + C_nZ_n^2]$$

where μ = ionic strength, C = molar concentrations, Z = valency and the subscripts a, b . . . n the various ionic species present in solution.

Ion-selective electrodes measure ion *activity* rather than *concentration.* However, we have no way by which the activity of a single ion in solu-

tion can be calculated or measured with any degree of accuracy. Therefore, Ca^{++} values can only be expressed in terms of concentration by comparison of samples with standard solutions of known composition (mM/L).

D. DISCUSSION

The demonstration by McLean and Hastings[81] that the ionized moiety of plasma calcium is the physiologically active fraction has stimulated search for a practical technique of Ca^{++} measurement applicable to biological media. The nomogram[83] based on empirical formulas for the base-binding properties of plasma proteins was derived by these authors on the asumption that the equilibrium relationship between protein-bound and ionized calcium is governed by the law of mass action. Currently available techniques for the quantitation of [Ca] include EDTA titration, photometry and atomic absorption spectrophotometry. In the normal individual, Ca^{++}_P values can be estimated indirectly from [Ca] and T.P. values (*cf.* p.29) but discrepancies as great as 0.20 mM/L between predicted and measured values have been encountered (fig. 5). Our data obtained in patients (figs. 28 and 35) indicate that [Ca] levels may not provide an adequate picture of disturbances in calcium homeostasis. This problem emphasizes the need for an accurate, reproducible and direct measurement of the physiologically active fraction of plasma calcium.

The bio-assay technique suggested by McLean and Hastings[81] is not practical, because of the variation in sensitivity toward Ca^{++}_P among the individual frog heart preparations; technical difficulties concerning perfusion (introduction of tubing into the ventricle) and recording (friction, inertia); and poor sensitivity at Ca^{++}_P values encountered clinically. McLean and Hastings have made the assumption that two solutions inducing identical response from the frog heart preparation contained identical Ca^{++} concentrations, other conditions being equal. In the case of biological fluids, it is impossible to prepare solutions of reference exactly comparable, particularly with respect to the presence of pressor substances.

The colorimetric Ca^{++}_P determination using murexide[31,125,140] requires ultrafiltration and removal of heavy metal ions prior to analysis; it is time consuming; its reproducibility is unsatisfactory and it is not suitable for routine clinical use. Some of the "normal" values obtained

with this technique were higher than those measured using either bioassay or electrode technique, a discrepancy explained by the variables introduced by ultrafiltration, the analytical method and possible contaminants.

The liquid membrane electrode has received much attention because of its selectivity toward calcium in the presence of other ions in biological media. The air-tight flow-through arrangement is most suitable for analysis of plasma as errors related to changes in pH secondary to loss of CO_2 are eliminated.

Appropriate interpretation of data obtained is contingent upon recognition of the limitation of the method employed. The electrode is ion-*selective* rather than ion-*specific*. The presence of other cations, therefore, imposes limitations on its sensitivity toward Ca^{++}. Errors due to the presence of Na^+ and K^+ ions are of importance particularly when Ca^{++} values are very low and errors due to H^+ are to be considered in fluids with pH below 5.5 (*cf.* p.25). As electrode response is logarithmic, small errors in potential generate rather large errors in apparent Ca^{++}. Recognition of this problem makes the investigator aware of the need for accurate electrode potential readings and electrode stability (*cf.* p.24).

CHAPTER II

MEASUREMENT OF IONIZED CALCIUM IN PLASMA OF NORMAL, ADULT MAN AND CONSTITUENTS WHICH INFLUENCE ITS CONCENTRATION

Data from the literature[85 108] have indicated a poor correlation between [Ca] and Ca^{++}_P both in volunteers and in patients with various diseases In normal volunteers Ca^{++}_P can be estimated reasonably well using the McLean-Hastings nomogram,[83] although a discrepancy as great as 0 2 mM/L between predicted and measured Ca^{++}_P may occasionally be found (fig 5) In patients a marked discrepancy between predicted and measured Ca^{++}_P values may be found (*vide infra*) In an attempt to clarify this discrepancy, we have measured P_I and [Mg] values in plasma obtained from these patients Subsequently we have investigated the magnitude of complexation *in vitro* by phosphate, lactate and citrate ions on the assumption that abnormal concentrations *in vivo* might explain the discrepancy encountered between [Ca] and Ca^{++}_P values A range of "normal" values for Ca^{++}_P , [Ca], T P , pH, P_I and [Mg] in plasma obtained from normal adult volunteers will be presented

A. IONIZED CALCIUM

1. Electrode system

The commercially available flow-through electrode system is not thermostated From the known temperature dependence of ionization constants and pH, we anticipated that temperature changes would have an effect on Ca^{++} measurement The sensing and reference electrodes were placed within a plexiglass box maintained at 37° C (fig 3) The thermostat probe and surface of the box were lined with a grounded copper mesh, making the system a Faraday cage as described by Sachs *et al* ,[130] shielding the sensing electrode from environmental electrical noise All samples obtained from normal volunteers were measured at 37° C We have found that in the range 25-37° C the effect of temperature is too small to warrant correction (*cf* p 49)

Accordingly, subsequent samples were analyzed with the electrode system at ambient room temperature.

2. Calcium standards

To establish the relationship between electrode potential, E (mV) and calcium activity, $A_{Ca^{++}}$ (mM/L), aqueous standard solutions with a concentration of 0.25, 0.50, 1.0 and 2.0 mM/L $CaCl_2$ were used to construct a calibration curve.[46, 99, 100] These solutions also contained 150 mM/L NaCl; their pH value fluctuated around 5.8. Data from the literature[85, 127] have demonstrated the lack of interference of hydrogen ions with Ca^{++} measurement in the pH span of 5.5 — 11 (*cf.* p.25).

The electrode was stored dry. Preliminary conditioning consisted of alternating the 1 mM/L standard solution with a random plasma sample until consecutive readings of the standard solution agreed within 0.1 mV. The three standards were then run through the electrode system and this procedure was repeated until each potential reading was stable within 0.1 mV. The minimum acceptable potential difference between consecutive standards was 5.5 mV as electrode sensitivity is impaired below that value.[46] Accordingly, we have tested the accuracy of an electrode with a potential difference of 5.5 mV against an electrode with a potential difference of 7.5 mV using aqueous solutions containing 0.75 or 1.50 mM/L $CaCl_2$ (with a background of 150 mM NaCl) and using plasma samples, Ca^{++}_P of which ranged from 0.60 to 1.30 mM/L. The differences between Ca^{++}_P measured by either electrode were not statistically significant. Readings of the absolute potential tended to drift with time when alternating plasma and aqueous solutions through the electrode. The magnitude of this drift was unpredictable and ranged from 0 to 3 mV. However, the slope between two consecutive Ca^{++} standard solutions remained constant. Greater electrode stability was achieved after the measurement of several plasma samples and with the intermittent use of a calcium chloride solution containing trypsin and triethanolamine (60 mg crystalline bovine pancreatic trypsin and 3 drops of 1 mM triethanolamine in 100 ml of an aqueous 2 mM $CaCl_2$ solution also containing 150 mM NaCl). It would appear that addition of trypsin removes protein from the membrane.[135] Triethanolamine is added to adjust the pH of the solution. Both these additives affect the Ca^{++} concentration of the calibration solution. Schwartz *et al.*[135] have found that Ca^{++} is lowered by binding to trypsin

while according to Lindgärde[75] Ca^{++} in aqueous $CaCl_2$ solutions containing triethanolamine were 10% lower than in pure $CaCl_2$ solutions. Therefore, the 2 mM $CaCl_2$ solution containing these additives was used intermittently for the purpose of stabilizing the electrode readings while purely aqueous $CaCl_2$ solutions with a background of 150 mM NaCl were used for calibration. All samples were measured in triplicate, preceded and followed by calibration with the 1 mM standard.

3. Preparation of blood samples

Ten ml of venous blood were collected from the antecubital veins of 32 normal adult volunteers into plastic syringes, the dead spaces (60 microliters) of which had been filled with a sodium heparin solution*. Heparin in a concentration of 6 I.U. per ml does not alter Ca_P^{++} and the change of volume is negligible (*cf.* Chapter III). The tips were covered with stainless steel caps.

The blood was centrifuged in the collection syringes with the tips up at 1500 *g* for 15 minutes and the separated plasma transferred anaerobically through a plastic 3-way stopcock into a second set of plastic syringes. This allowed us to collect and handle the blood without significant loss of CO_2 up to the time of measurement and proved more practical than collection under oil or in heparinized stoppered tubes. Collection under oil does not always prevent CO_2 loss and, more important, contamination of the electrode with small amounts of oil interferes with Ca_P^{++} measurement. The plastic does not alter Ca_P^{++} ; we have found that Ca_P^{++} values do not change even after 6 passages from one syringe to another. This contrasts favorably with the findings of Oreskes *et al.*[99] and of Sachs *et al.*[130] who noted a progressive decrease of Ca^{++} with passage of serum from one siliconized glass tube to another.

4. Reproducibility

Reproducibility was tested over the range encountered clinically. Increasing amounts of $CaCl_2$ (100, 200 or 400 μl of a 25 mM $CaCl_2$ solution) were added to 5 ml aliquots of heparinized whole blood (collected from a normal adult volunteer) decalcified by passage over an ion-exchange resin (*cf.* p.35). [Ca] values in plasma prepared in

* "Liquaemin" (1000. I.U. per ml) Organon, West Orange, N.J.

this manner were 1.45, 2.87 and 4.23 mM/L, respectively. Addition of these amounts of $CaCl_2$ would have made [Ca] values of these samples 1, 2 or 3 mM/L if $CaCl_2$ were equally distributed over plasma and red blood cells. This is evidently not the case; as a matter of fact $CaCl_2$ added to a whole blood sample is only minimally taken up by red blood cells. The measured [Ca] values agree closely with values predicted from the amount of $CaCl_2$ added and plasma volumes at a hematocrit of 39%. Values for each of these samples were determined ten times. Each value was the average of three readings. The results are presented in table 1. These observations are in agreement with other reports in the literature.[75, 85, 99, 112] Moore[85] has emphasized the need for accurate electrometer readings. For example, at Ca_P^{++} = 1 mM/L, an error of 1 mV potential difference would lead to an error in Ca_P^{++} of approximately 10%. The accuracy of electrode potential readings was within 0.2 mV as recalibration of the electrode was done preceding and following each plasma sample.

[Ca] (mM/L)	N	1,45	2,87	4,23
Ca_P^{++} (mM/L) *	10	0,67 ± 0,03	1,34 ± 0,04	2,27 ± 0,04
Coefficient of Variation (%)		4,1	2,8	1,7

* M ± S.D.

Table 1. Reproducibility of Ca_P^{++} measurements. Increasing amounts of $CaCl_2$ were added to decalcified whole blood and resulting Ca_P^{++} measured in triplicate on ten different occasions. The coefficient of variation decreased with increasing Ca_P^{++}.

The standard deviation was small (0.028 at Ca_P^{++} = 0.67 mM/L and 0.04 at Ca_P^{++} = 2.27 mM/L) so that the coefficient of variation de-

creased with increasing Ca^{++}. Coefficient of variation (C.V.) is defined by the following formula:

$$C.V. = \frac{S.D.}{M} \times 100\%$$

where S. D. = standard deviation and M = mean (*cf* p.28)

5. Ion selectivity

The degree to which electrode potentials represent a particular ion in preference to another is expressed by *selectivity constants.* Although several recent publications[3, 66, 112, 130, 138] have described the calcium electrode as *specific,* it is in fact only *selective.* The response of the pH electrode to sodium ion activity at a low hydrogen ion concentration is an example of this problem.

The potassium selectivity constant of the calcium electrode is equal to that concentration of Ca^{++} which generates the same potential as 1 M potassium. Cations are expected to generate an electrode response which interferes with Ca^{++} measurement if the selectivity constant for these ions is high. The error caused by interfering ions depends on their *activity* and on the *selectivity coefficient* of the electrode. This can be expressed by the equation:

$$\text{error} = C \cdot \frac{A_{I+}}{A_{Ca^{++}}}$$

where C = selectivity coefficient, A = activity of the interfering (I^+) and calcium (Ca^{++}) ions respectively.

Ross[127] and Moore[85] have indicated that in aqueous $CaCl_2$ solutions, the interference with Ca^{++} measurement caused by hydrogen ions is negligible in the pH range 5.5 - 11. This is important since the pH values of the aqueous calibration solutions generally fluctuate around 5.8. Thus exposure of the membrane to plasma (pH in the physiological range) alternating with calibration standards (pH 5.8) is not expected to introduce significant error. Selectivity constants for sodium and potassium are of the magnitude of 10^{-3} so that potassium concentrations normally present in serum cause little or no interference. However, the sodium effect is not negligible, primarily because of its concentration. To account for this influence, sodium chloride is added to the standard solutions to a final sodium concentration of 150 mM, a maneuver which serves to increase the ionic strength of the calibration

standards to a value approximately equal to that of plasma. A significant difference in ionic strength between the calibration standard and the sample may generate a parasite potential ("junction potential") between the sample solution and the solution in the external reference electrode.[130] The variation in junction potential may be unpredictable, particularly when the aqueous calibration solutions move past the electrode alternately with protein-containing solutions. Variation in ionic strength between 0.12 and 0.16 produce only negligible effects on the junction potential.[130]

Magnesium is another divalent ion of clinical importance. Its serum concentration is approximately half that of calcium. Data from the literature[100, 116, 130] have suggested an electrode selectivity constant on the order of 0.014. In other words, at a [Mg] concentration within the physiological range, the error in Ca^{++} value is on the order of 1%. Unphysiologically high [Mg] concentrations give inappropriately low readings for Ca^{++}_P (*cf.* p.53).

Anions, particularly phosphate, bicarbonate, citrate and lactate would be expected to lower calcium activity by complexation, chelation or ion-pair formation. Indeed, we have found that when present in sufficient concentrations, these anions can significantly lower Ca^{++} *in vitro* (*cf.* p.54).

Perchlorate in concentrations over 10^{-3} M is reported[100] to interfere with Ca^{++} measurement. Thus, Ca^{++} cannot be measured following deproteinization of serum samples using perchloric acid.

6. Lower limit of detection

Sensitivity of the electrode at low Ca^{++} concentrations is limited by the presence of sodium ions as outlined above and by the water solubility of the liquid ion-exchange resin, which dissolves in the solution moving past the electrode and dissociates to form calcium ions. This back ground level of calcium is on the order of 0.005 mM/L. Ross[127] has reported that the electrode response follows the behavior predicted by the Nernst equation down to a Ca^{++} concentration of 0.05 mM/L.

B. TOTAL CALCIUM

Total calcium ([Ca]) values were determined in duplicate by ethylenediaminetetraacetate (EDTA) titration using calcein (fluorescein-methyleneiminodiacetic acid) as an indicator.[9] This assay is based

on the fluorescence of calcein in the presence of calcium. Moore[85] has demonstrated a close agreement between [Ca] values determined by EDTA titration and atomic absorption spectrometry. In the titration method, EDTA is used as a quantitative chelating agent for calcium, the end-point being the quenching of calcein fluorescence. Although the end-point is subjectively determined, it is well reproducible by the same observer. All determinations in our study were done by the same individual. [Ca] values were calculated from the ratio of the amount of EDTA necessary to quench the fluorescence of the plasma sample and the amount necessary to reach the same end-point with a standard solution containing calcium chloride (2 mM/L).

C. INORGANIC PHOSPHATE

Inorganic phosphate (P_I) values were measured according to a modification of the method described by Chen *et al.*[20] using 1.93% (w/v) sodium molybdate and 3.5% (w/v) ascorbic acid in 0.6 M sulfuric acid. After deproteinization of the plasma samples using 6% perchloric acid and incubation (50°C) the reduced phosphomolybdate was measured at 820 mμ spectrophotometrically*. P_I values were calculated by comparing the optical density measured in the sample with the optical density measured in two solutions containing 0.5 or 1.0 mM/L phosphate, respectively.

D. TOTAL MAGNESIUM

Total magnesium ([Mg]) concentrations were measured using the method described by Garner[36] using titan yellow as an indicator. Magnesium is precipitated as the hydroxide in the presence of titan yellow which changes to a red color. Poly (vinyl-alcohol) was used as a stabilizing agent. The intensity of the color was measured spectrophotometrically at 540 mμ and [Mg] values were calculated by comparing the optical density measured in the plasma samples with the optical density measured in two standard solutions containing magnesium chloride (1 or 3 mEq/L).

* Beckman, S. Pasadena, Calif.

E. TOTAL PROTEIN

Total protein (T.P.) values were measured using a refractometer* according to the method described by Sunderman.[145]

F. pH

pH was measured using a thermostated micro glass electrode and a reference electrode.**

G. HEMODYNAMIC PARAMETERS

Arterial and central venous blood pressures were recorded continuously using a 4 channel recorder*** and appropriate transducers attached to indwelling catheters. Cardiac output determinations were made using dye dilution technique (indocyanine green); the dye was injected into the central venous pressure catheter. The dye curve was recorded by a Cardio-Densitometer•. Systemic vascular resistance (SVR) was calculated according to the following formula:[141]

$$SVR = \frac{MAP - RAP}{C.O.} \times 1.332 \qquad (dyn \cdot sec \cdot cm^{-5})$$

where MAP = mean arterial blood pressure (torr), RAP = right atrial blood pressure (torr) and C.O. = cardiac output (ml/sec).

H. STATISTICAL EVALUATION OF DATA

Values for mean and standard deviation were calculated using a programmed computer••. The program for calculation of mean ($\bar{x}$) and standard deviation (S.D.) incorporated the following formulae:

$$\bar{x} = \frac{\Sigma x}{n} \text{ and } S.D. = \sqrt{\frac{\Sigma(x^2 - n\bar{x}^2)}{n-1}}$$

where Σ = algebraic sum; n = number of samples; x = variable under consideration; and $\bar{x}$ = mean of variable. Significance of the changes from control in one group of patients was determined with the paired *t*-test; the significance of the differences between the means of two groups of patients with the unpaired *t*-test.

* American Optical, Buffalo, N.Y.
** Radiometer PHM 27, Copenhagen, Denmark
*** Sanborn, Hewlett Packard, Waltham, Massachusetts
• Beckman, Fullerton, Calif.
•• Olivetti Underwood

I. RESULTS AND COMPARISON WITH DATA FROM THE LITERATURE

In table 2 values for Ca^{++}_P, [Ca], P_I, T.P., [Mg] and pH measured in venous blood obtained from 32 normal adult volunteers (22 male and 10 female, age range 19-46 years) and from 20 children (age range: 2-12 years) are presented. In normal adult volunteers, measured

	N	Ca^{++} (mM/L)*	[Ca] (mM/L)*	pH *	P_I (mM/L)*	[Mg] (mEq/L)*	T.P. (g/100 ml)*
Normal adult Volunteers (age range: 19 - 46 y)	32	1.01 ± 0.07	2.34 ± 0.197	7.37 ± 0.05	0.95 ± 0.137	2.10 ± 0.25	7.31 ± 0.51
Children (age range 2 - 12 y)	20	0.86 ± 0.10	2.24 ± 0.08	7.35 ± 0.05	0.98 ± 0.33	2.04 ± 0.20	6.73 ± 0.42
p		< 0.01	<0.01	N.S.	N.S.	N.S.	< 0.01

* Mean ± S.D.

Table 2. Values measured in 32 normal adult volunteers (awake) and in 20 children (during induction of inhalation anesthesia). The p values are relative to normal volunteers.

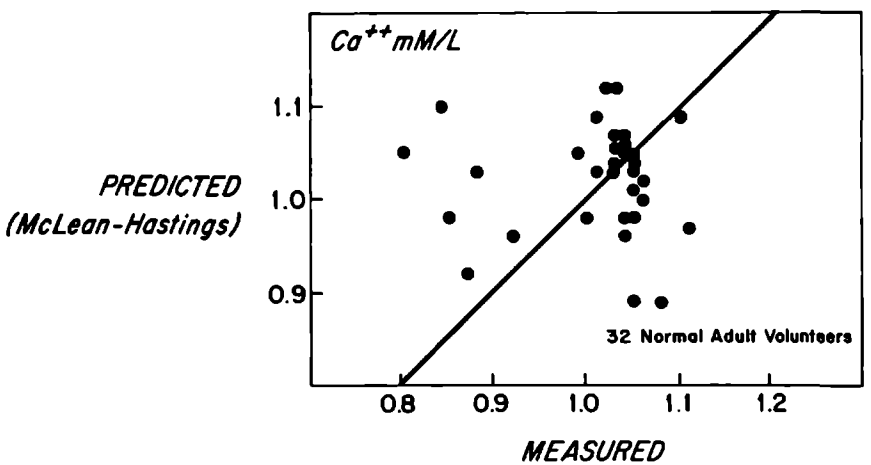

Fig. 5 — Scattergram showing the relationship between measured Ca^{++}_P values in 32 adult normal volunteers and those predicted from the McLean-Hastings nomogram. The solid line represents the line of perfect agreement. An independent variation within the range was apparent, although there was no significant ($p > 0.1$) difference between the means of predicted and measured values.

Ca^{++}_P values were not significantly different ($p > 0.1$) from those predicted from the McLean-Hastings nomogram (fig. 5); however, within the normal range, predicted and measured Ca^{++}_P values varied independently indicating a poor correlation between [Ca] and Ca^{++}_P. This is in agreement with data presented by Moore[85] and by Pittinger.[108] Ca^{++}_P values measured in venous blood obtained from 20 children during induction of inhalation anesthesia using halothane in oxygen in preparation for elective tonsillectomy or eye surgery were lower than in adult normal volunteers and lower than predicted (fig. 6 and table 2). These differences were statistically significant ($p < 0.01$).

A selection of "normal" Ca^{++}_P values reported in the literature is presented in table 3.

J. DISCUSSION

Critical survey of the literature (table 3) reveals large variation in Ca^{++}_P with reported values in the range of 0.94 to 1.27 mM/L. Authors reporting these extremes claim a standard deviation of approximately

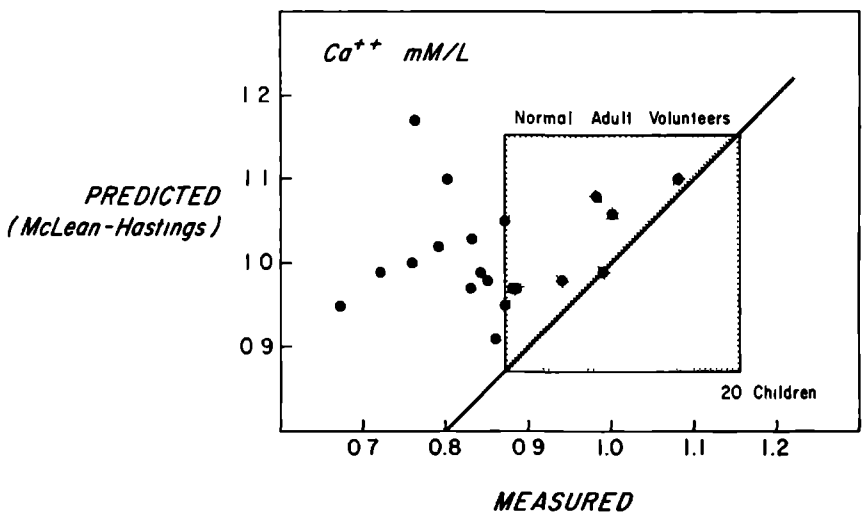

Fig. 6 — Relationship between Ca^{++}_P measured and predicted from the McLean-Hastings nomogram in 20 children undergoing elective tonsillectomy or eye operation. Measured Ca^{++}_P concentrations were significantly lower than predicted and lower than those found in adult normal volunteers. Blood samples were drawn from the antecubital veins during induction of inhalation anesthesia using halothane in oxygen. The shaded area indicates the 95% confidence limits of Ca^{++}_P measured in normal volunteers.

Author	N	Ca^{++} (mM/L)*	[Ca] (mM/L)*	Remarks
		Bio-essay		
McLean-Hastings	10	1.25 ± 0.07	2.48 ± 0.34	Frog Heart
Morison	12	1.16 ± 0.12	2.80 ± 0.18	Frog Heart
		Colorimetric Determinations		
Cham	18	1.10 ± 0.049	2.67 ± 0.06	Plasma, Autoanalyzer
Fanconi	7	1.47 ± 0.22	2.36 ± 0.28	Plasma
Lumb	10	1.46 ± 0.14	2.43 ± 0.26	Plasma
Pedersen	32	1.13 ± 0.03	2.32 ± 0.08	Ultrafiltrate
Putman	12	1.10 ± 0.07	2.39 ± 0.10	Ultrafiltrate
Rose	7	1.46 ± 0.04	2.60 ± 0.07	Ultrafiltrate
Schirardin	13	1.12 ± 0.09	2.44 ± 0.08	Ultrafiltrate
		Electrode Measurements		
Arnold	27	1.26 ± 0.16	2.43 ± 0.14	Dip Electrode
Arras	30	1.11 ± 0.06	Not Specified	
Hansen	35	1.10 ± 0.04	2.38 ± 0.08	Patients
Harris	68	1.17 ± 0.03	2.55 ± 0.07	
Hattner	23	0.99 ± 0.08	2.45 ± 0.19	
Ladenson	86	1.27 ± 0.05	2.42 ± 0.075	
Li-Piechocki	397	1.22 ± 0.045	2.29 ± 0.12	
Lindgärde	297	1.11 ± 0.05	Not Specified	
Moore	19	1.16 ± 0.05	2.47 ± 0.29	37° C
Oreskes	50	1.21 ± 0.11	2.65 ± 0.21	Dip Electrode
Pittinger	42	1.08 ± 0.05	2.48 ± 0.10	
Radde	13	1.18 ± 0.15	2.41 ± 0.27	
Robertson	21	1.23 ± 0.03	2.43 ± 0.07	37° C
Robertson and Piecock	47	1.24 ± 0.08	2.39 ± 0.07	Ultrafiltrate
Sachs	22	1.10 ± 0.20	2.41 ± 0.20	Children, Age 5-12
	13	1.04 ± 0.10	2.46 ± 0.06	Adults
Schwartz	30	0.96 ± 0.045	Not Specified	
Seamonds	84	1.08 ± 0.03	2.50 ± 0.08	37° C
Studer	40	1.26 ± 0.035	2.42 ± 0.15	

* Mean ± S.D.

Table 3. Selected "normal" values reported in the literature. Values obtained by bio-assay were expressed in mM/kg. Unless otherwise specified, all measurements were made using the flow-through electrode at ambient room temperature on serum samples prepared from venous blood obtained from normal adult volunteers.

8% for the presented material. Analysis of these reports reveals that the values were measured in serum or plasma obtained from normal adult volunteers and occasionally from patients claimed to have no abnormality in calcium metabolism. Our data suggest that measurements in blood obtained from patients cannot be used to establish a range of normal. Berliner *et al.*[7] have described that elevation of Ca_P^{++} may be observed in patients secondary to immobilization. Comparison of our mean Ca_P^{++} values (table 2) with those reported in the literature reveals that our data agree closely with those of several authors,[46, 130, 135] but are lower than the values reported by others. This difference is probably due to differences in protocol and technique. According to Schwartz *et al.*[135] addition of trypsin and triethanolamine to the calibration standards introduces a systematic error by increasing the sample Ca_P^{++} values by about 10%. To avoid these errors in measurement, our calibration standards did not contain these additives.

Van Leeuwen[69] has called attention to the fact that upper arm compression employed to induce venous congestion prior to venepuncture may produce a substantial increase in T.P. Therefore simultaneous increases in both [Ca] and Ca_P^{++} might be expected if the law of mass action applies. Indeed, Van Leeuwen[69] has measured increases of [Ca] values by as much as 40% following venous stasis. This consideration is important particularly in the evaluation of those studies involving normal volunteers selected among blood donors in a blood transfusion service. We have taken samples from antecubital veins without venous stasis in each volunteer.

Diurnal variation in inorganic phosphate has been reported.[156] Although these changes may have an effect on Ca_P^{++} they are probably of minor importance.

Regional differences in response to drugs have been observed. For example, Katz[57] has found significant differences in response to muscle relaxants between patient populations in London, England and New York, N.Y. It is possible, then, that there are differences in Ca_P^{++} between normal volunteers studied at different locations.

"Normal" Ca_P^{++} values for volunteers of the pediatric age group are not available. Available data[130] were obtained from children admitted to the hospital for various medical reasons. We were interested in establishing a range of Ca_P^{++} values in pediatric patients in whom the least disturbance in calcium metabolism might be expected. There-

fore we have chosen children admitted for an elective tonsillectomy or eye surgery, bearing in mind that these values were obtained under general anesthesia. The samples were taken from the antecubital veins within 3 minutes following induction of inhalation anesthesia using halothane in oxygen. Ca^{++}_{P} values in these patients were significantly ($p < 0.01$) lower than those measured in normal adult volunteers. These findings are consistent with data presented by Pittinger[108] showing that similar changes occurred in adults following induction of anesthesia using thiopental. It is possible that the low values measured in these patients are the result of interaction between Ca^{++}_{P} and the anesthetic agent or the effect of changes in hemodynamics induced by premedication and the anesthetic on calcium homeostasis (*cf.* p.86).

Because of systematic errors introduced by differences in protocol and technique it is desirable that a set of "normal" values be established for each parameter measured. This approach permits meaningful evaluation of values measured in patients with various disease states.

CHAPTER III

EFFECTS OF COAGULATION AND HEPARINIZATION

Data from the literature[3, 4, 43, 44, 85] have suggested that Ca^{++} should be measured in serum because of the potential error when heparin is added as an anticoagulant. Other investigations have dealt with the determination of Ca^{++} in plasma[99, 112, 132, 144] using stoppered glass tubes prefilled with crystalline heparin. Moore[85] has indicated that addition of increasing amounts of heparin to an aqueous solution containing 5 mM/L $CaCl_2$ progressively decreased Ca^{++} concentration. However, the heparin concentrations used in his experiments were far above those required clinically; in addition, this effect was demonstrated in aqueous solutions not containing protein, and no mention was made of the concentration change resulting from the volume of added heparin.

None of the investigators advocating serum as a suitable medium for Ca^{++} measurement have shown that Ca_S^{++} represents the amount present in whole blood on the assumption that no significant quantity of calcium is lost during the clotting process. We have, therefore, evaluated the effect of coagulation and heparinization on Ca^{++} particularly in view of our intention to measure Ca^{++} in blood samples collected for blood gas analysis.

A. EFFECT OF HEPARIN

The practical advantage of measuring Ca^{++} in heparinized blood led us to investigate the effects of heparin. We have found that 2 I.U. per ml whole blood is sufficient for effective anticoagulation lasting several hours. Clinically, 60 I.U. heparin are added to 10 ml whole blood for blood gas determination. The authors[85, 105, 130] who have reported a decrease of Ca_P^{++} upon addition of heparin used evacuated glass tubes prefilled with an unknown amount and type of crystalline sodium heparin.

Four normal volunteers donated venous blood which was collected in plastic syringes containing glass beads to promote coagulation. The samples were then centifuged at 1500 *g* for ten minutes. The sera were transferred into other plastic syringes which were closed with 3-way stopcocks. Each serum sample was divided into 4 aliquots; Ca^{++}_s was measured in native serum and in serum to which 0.5, 1.0 or 2.0 m$\underline{M}$/L $CaCl_2$ had been added, with and without heparin. By adding various amounts of $CaCl_2$ to serum, the effects of heparinization could be studied over a Ca^{++} range including that encountered clinically. To accomplish this, a quantity of 100, 200 or 400 microliters of a 25 m$\underline{M}$ $CaCl_2$ solution was pipetted into the barrels of a third set of plastic syringes. The barrels were then placed in an oven at 70° C in order to evaporate the solution. This technique eliminated errors related to changes of volume. To one half of the syringes prefilled with $CaCl_2$ we added 50 microliters of a sodium heparin solution*. The solution was evaporated by placing the barrels in the oven at 70° C again. In the next section (*cf.* p.39) we will demonstrate that the activity of heparin was not lost by exposure to this temperature. To the other half of the syringes no heparin was added. All syringes were then filled with 5 ml of serum prepared in the manner described above.

The results are presented in figure 7. There was close agreement between Ca^{++}_s values measured in serum with and without heparin.

B. COMPARISON OF IONIZED CALCIUM IN SERUM AND PLASMA

In order to determine whether Ca^{++} concentrations are significantly different in serum and plasma, measurements were made in serum and plasma prepared from the same blood specimen. An ion-exchange resin (Dowex 50) was used to remove calcium from the blood.[155] The ion-exchange column is contained in an in-line filter connecting a 15-gauge needle with a blood collection bag. Two 3-way stopcocks were inserted in the plastic tubing at either side of the ion-exchange column (fig. 8), thus permitting sequential washings of the resin with 1 $\underline{M}$ and 150 m$\underline{M}$ NaCl solutions. With this washing procedure preceding the passage of blood, small resin beads which would have entered the blood collection bag were removed. Saturation of the resin with sodium was achieved using 1 $\underline{M}$ NaCl solution and excess sodium was removed

* "Liquaemin" (1000 I.U. per ml) Organon, West Orange, N.J.

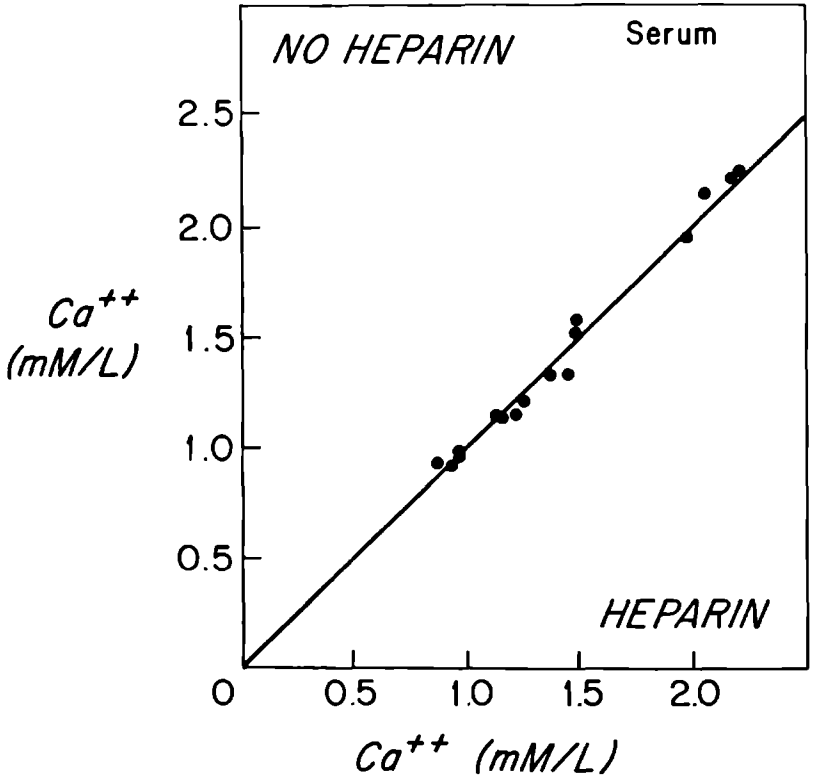

Fig. 7 — Effect of heparinization. Increasing amounts of $CaCl_2$ were added to serum with and without heparin (50 I.U. per 5 ml serum) so that the effect of heparin could be studied over the range of Ca^{++}_P encountered clinically. There was close agreement between Ca^{++}_P values measured in native and heparinized serum.

using 150 mM NaCl solution. Therefore, hemolysis upon contact of the blood with the column did not occur.

Arterial blood was obtained from 3 patients who had existing arterial lines for continuous blood pressure monitoring or for repeated arterial blood sampling. The 15-gauge needle of the collection system was connected to the arterial line through an obturator, and permitted blood to pass over the ion-exchange resin before entering the plastic blood collection bag. The first 30 ml of blood were discarded. As the blood was not exposed to air during collection, no appreciable loss of CO_2 oc-

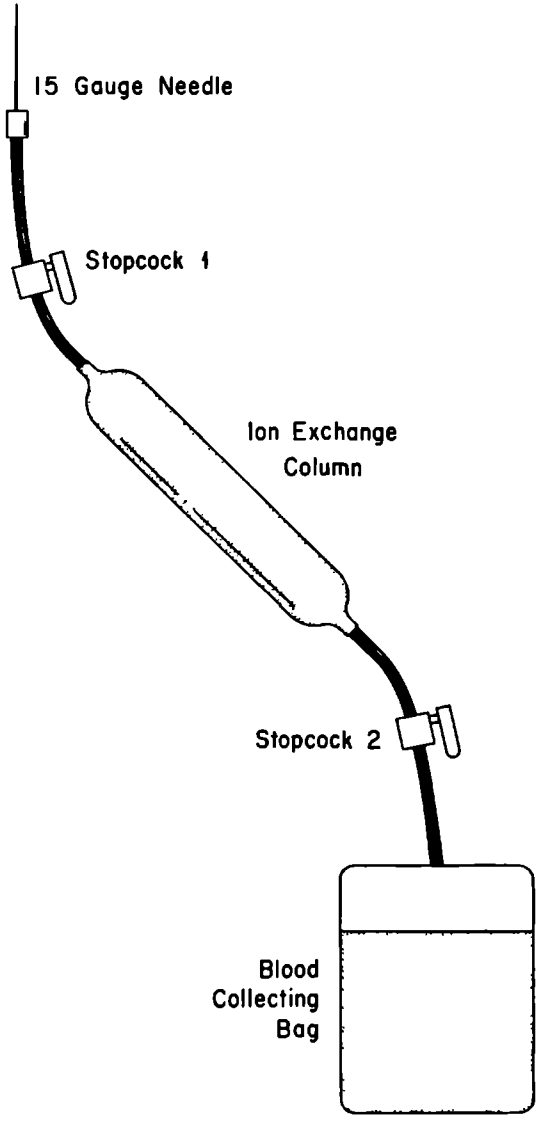

Fig. 8 — Collection system used for the preparation of calcium free whole blood. At both sides of the in-line filter containing the ion-exchange resin, a 3-way stopcock was placed in order to permit sequential washing of the resin using 1 M NaCl (for saturation) and 0.150 M NaCl (for removal of excess sodium). The first 30 ml of whole blood were collected in a syringe attached to stopcock 2 and discarded followed by collection of decalcified unadulterated whole blood in the plastic bag.

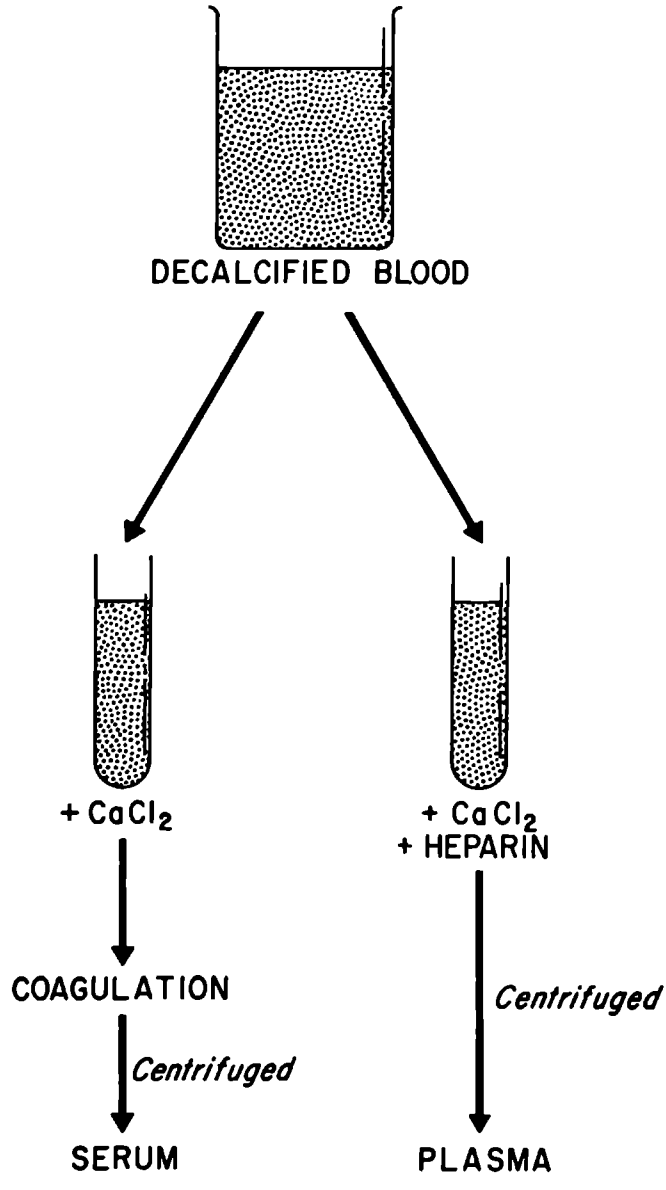

Fig. 9 — Diagram showing the preparation of plasma and serum from one aliquot of whole blood. Coagulation took place promptly even at [Ca] levels of 0.25 mM/L, while 50 I.U. heparin per 5 ml whole blood were effective in preventing coagulation following addition of $CaCl_2$ to decalcified whole blood.

curred. Decalcification prevented coagulation and the former was verified by direct measurement: levels of [Ca] and Ca^{++} were below detection limits by atomic absorption spectroscopy and electrode potentiometry, respectively. These data are in contrast to those of Loeb[77] who has suggested that serum proteins can be rendered calcium-free only at a pH of 2.5 or below. His findings, however, were based on dialysis experiments.

Use of the ion-exchange resin does not remove calcium alone; magnesium and potassium are also exchanged for sodium and the plasma sodium concentration increases an average of 8 mEq/L with this procedure.

Subsequent processing of the decalcified blood is depicted in figure 9. Addition of $CaCl_2$ and heparin to syringes was accomplished according to the technique previously described (*cf.* p.35).

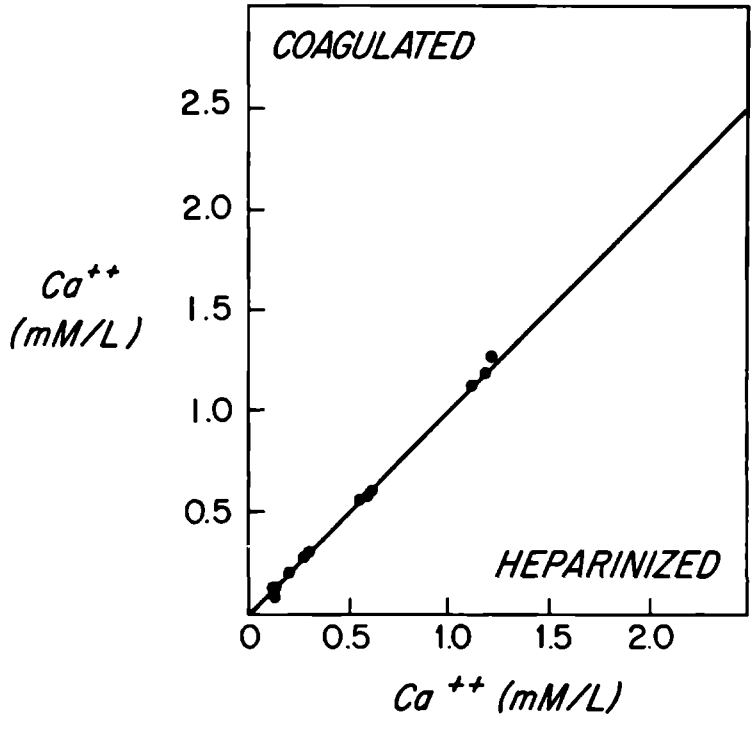

Fig. 10 — Relationship between Ca^{++} in serum and plasma, prepared from one aliquot of whole blood as shown in fig. 9. The solid line represents the line of perfect agreement.

Upon addition of 5 ml of decalcified blood to syringes containing CaCl and heparin that had been exposed to a temperature of 70° C, no clotting was observed for several hours, while clotting took place promptly in those syringes containing $CaCl_2$ only This suggests that exposure of heparin to 70° C does not significantly alter its activity

All syringes were centrifuged at 1500 *g* for 15 minutes, serum or plasma was transferred using 3-way stopcocks into other syringes and Ca^{++} values measured The results are presented in figure 10 There was close agreement between serum and plasma Ca^{++} values

C. COMPARISON OF IONIZED CALCIUM IN PLASMA AND WHOLE BLOOD

Ten ml of arterial blood were collected from each of 22 patients recovering from abdominal or parathyroid surgery The specimens were heparinized by prefilling the dead spaces of the syringes with a heparin solution* The dilution effect due to this amount of heparin in 10 ml of whole blood is negligible About 5 ml of whole blood were transferred into another syringe which was placed on ice, the remaining 5 ml were centrifuged and the plasma separated Ca^{++} values were measured in whole blood which was first allowed to reach room temperature, and in plasma Determinations of pH were done prior to Ca^{++} determinations and when necessary, pH values in whole blood and plasma samples were equalized by tonometry with 8% CO_2/92% O or air, respectively on the assumption that not necessarily pCO_2 but pH is the determining factor in the calcium binding of proteins Indeed, Lindgarde,[35] Gupta[41] and Loken[38] have shown that the effects of pH changes induced by addition of HCl (1 M) or NaOH (1 M) and those induced by changes in pCO_2 (tonometry) on Ca^{++} were identical The duration of tonometry was determined by serial pH measurements, it was our purpose to yield pH values in the range encountered clinically and therefore, tonometry was limited to arbitrary pH values ranging from 7 15 to 7 55 According to figure 11 there was close agreement between Ca^{++}_{I} and Ca^{++}_{WB} ($r = 0\ 981$)

We may conclude that Ca^{++} in both serum and plasma represents Ca^{++} in whole blood, calcium plays a catalytic role and minimal

* Liquaemin (1000 I U per ml) Organon, West Orange, N J

quantities are consumed by the clotting process. Moore[85] has found in 6 normal adult volunteers that Ca^{++} values were generally lower in heparinized whole blood than in serum at physiological pH; however, this difference was small (0.045 mM/L at pH 7.32).

Use of whole blood for measuring Ca^{++}_{WB} is limited by the time required to obtain a reproducible electrode potential (at times 15 minutes). The reason for this is not immediately clear. In addition, metabolism of the red cells continues, and pH of plasma may decrease. Sachs *et al.*[130] have emphasized that the sample must reach room temperature

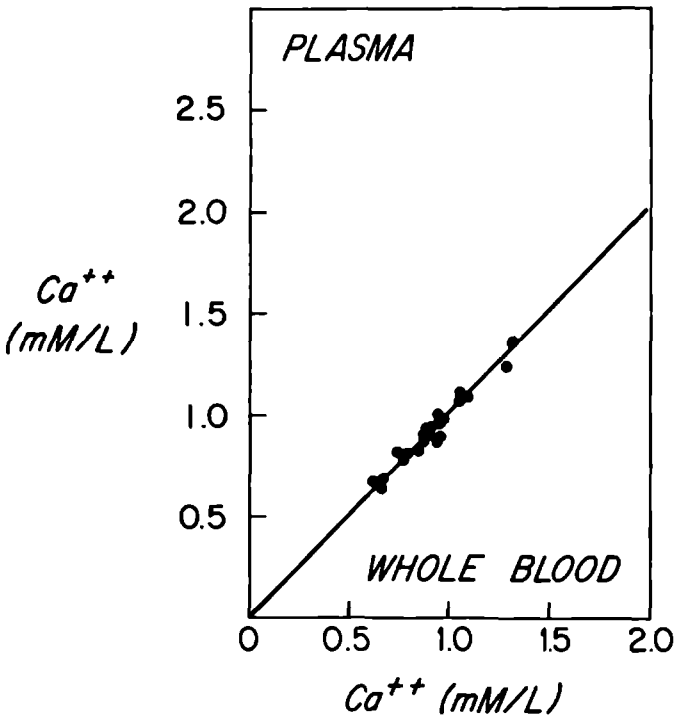

Fig. 11 — Relationship between Ca^{++} values measured in plasma and whole blood. Heparinized blood samples were taken from 22 patients recovering from abdominal or parathyroid surgery. The samples were divided into two aliquots. One was centrifuged and plasma was prepared, the other half was kept on ice. Ca^{++} values were then measured in plasma and in whole blood that had first reached room temperature; pH values were equalized by tonometry (*cf.* p. 40) when necessary. The solid line represents the line of perfect agreement.

prior to measurement as a difference in temperature between the sample and the KCl solution in the external reference electrode can create a junction potential.

Measurement of Ca^{++} is presently done in plasma at ambient room temperature although determination in whole blood would be ideal. Further developments in electrode technology may permit measurement in whole blood in the future.

D. EFFECT OF HEPARIN CONCENTRATION

The effect of heparin concentration on Ca^{++} was determined by adding increasing amounts of heparin to initially decalcified whole blood (using an ion-exchange resin, *cf.* p.35) to which $CaCl_2$ (0.75 or 1.95 mM/L) and heparin were subsequently added. The technique employed for achieving the different Ca^{++}_P levels has been described previously (*cf.* p.35). In this fashion the effects of various heparin concentrations were studied at two different Ca^{++}_P levels. These studies were carried out with a heparin solution prepared from purified crystalline sodium heparin* and a commercially available heparin solution* containing benzyl alcohol as a preservative. Both solutions were evaporated by

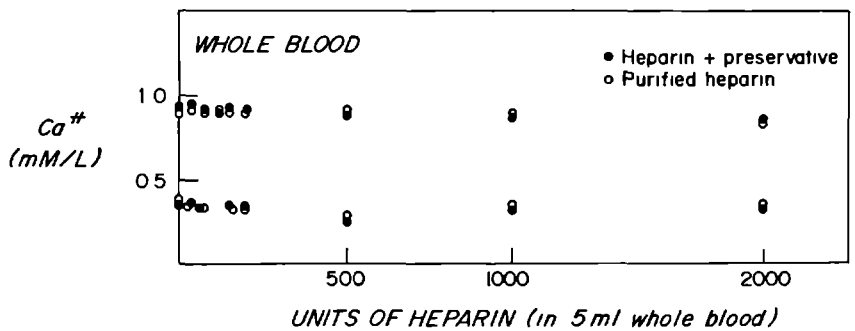

Fig. 12 — Effect of heparin concentration on Ca^{++} *in vitro*. Increasing amounts of heparin with and without preservative were added to two aliquots of plasam, in which [Ca] had been made 0.75 and 1.95 mM/L respectively by adding crystalline $CaCl_2$ to previously decalcified whole blood (Ca^{++}_P was 0.38 and 0.89 mM/L respectively). The heparin solution was added to an empty syringe barrel and subsequently evaporated prior to filling with whole blood. Up to 2000 I.U. heparin per 5 ml whole blood did not significantly alter Ca^{++}_P.

*Organon. West Orange, N.J.

placing the syringe barrels in an oven at 70° C. The effect of exposure of heparin to this temperature has been mentioned previously (*cf.* p.39). The results are presented in figure 12. Mean Ca^{++}_{P} values in samples with [Ca] values of 1.95 mM/L to which heparin with or without preservative had been added were 0.89 ± 0.02. At a [Ca] level of 0.75 mM/L mean Ca^{++}_{P} values were 0.34 ± 0.02 mM/L.

E. EFFECT OF SYSTEMIC HEPARINIZATION

The *in vitro* effect of heparin was expected to apply *in vivo,* but there are no data available to support this hypothesis. We have, therefore, evaluated the *in vivo* effect of heparin on Ca^{++}_{P} by measurements made just prior to and 3 minutes following the intravenous administration of heparin (3 mg per kg of body weight) to 12 patients prior to cardiopulmonary bypass for open heart surgery. During this 3 minute period, the infusion of fluids was kept at a minimal rate (25 ml per hour) and ventilation was maintained at a constant depth and rate. The amount of heparin administered corresponds to approximately 5 I.U. per ml whole blood, assuming a total circulating blood volume of 70 ml per kg of body weight. In cases where a difference in pH had occurred between the sample taken before and that taken after heparinization, pH adjustment was made by tonometry with 8% CO_2 or air (*cf.* p.40). The results are presented in figure 13. There was close agreement between Ca^{++}_{P} before and after systemic heparinization ($r = 0.952$).

F. DISCUSSION

Most authors have described Ca^{++} values measured in serum because of potential error introduced when heparin is used as an anticoagulant on the assumption that no significant amount of Ca^{++} is lost during the clotting process. Other investigators have indeed reported that a lowering of Ca^{++} occurs secondary to addition of heparin.

Heparin is a sulfated mucopolysaccharide with a strongly negative charge which might be expected to lower Ca^{++} by complexation. Indeed, our findings have confirmed data presented by Moore[85] who observed a progressive lowering of Ca^{++} in an aqueous $CaCl_2$ solution to which increasing amounts of heparin had been added. These data are not necessarily applicable to serum or plasma, primarily because of charges on proteins that may in part neutralize those on the heparin molecule. Unfortunately, changes of volume secondary to addition of

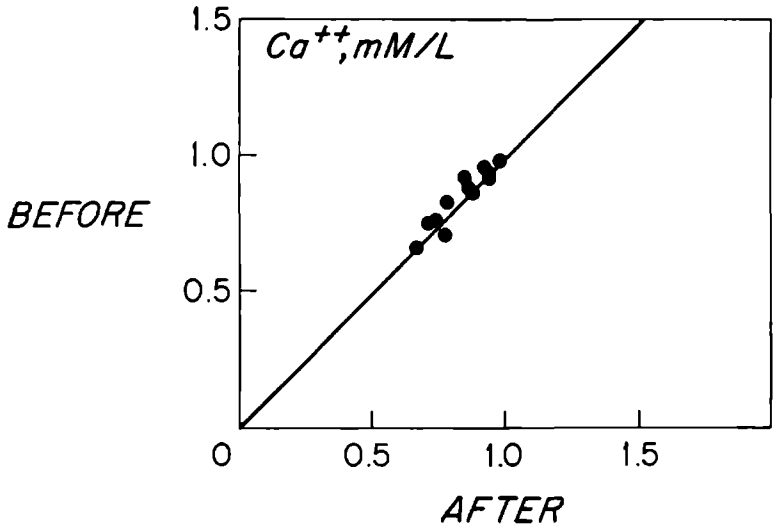

Fig. 13 — Effect of systemic heparinization. Blood samples were drawn just prior to and 3 minutes following systemic heparinization in 12 patients in whom cardiopulmonary bypass was necessary in preparation for open heart surgery. Heparinization was accomplished using a heparin dose of 3 mg per kg of body weight. A close agreement was found between Ca^{++}_P values measured before and following heparinization.

heparin solution to the aqueous $CaCl_2$ solutions were not specified in Moore's data. Other investigators have compared serum and plasma prepared from venous blood samples collected almost simultaneously in stoppered siliconized glass tubes with or without crystalline heparin. The amounts and type of heparin were not specified by these investigators. Silicone has been reported[85] to induce changes in Ca^{++}_P while incomplete filling of these glass tubes may be associated with pH changes due to release of CO_2 into the gas phase. Some investigators indicate that these glass tubes were chilled on ice prior to Ca^{++} measurement. The possible temperature difference between sample and KCl solution in the external reference electrode was not specified. The importance of this temperature difference has been mentioned previously (*cf.* p.41).

Definition of the possible error introduced by heparin would permit Ca^{++} measurement in whole blood or plasma collected for blood gas analysis by application of a correction factor. However, heparin added

in the quantities necessary for effective anticoagulation did not alter Ca^{++}_P concentration in the physiological range (*cf.* p.42).

To clarify the magnitude of change in Ca^{++} secondary to the clotting process, there was a need for comparison between serum and plasma of otherwise identical composition. In our experience, an ion-exchange resin (Dowex 50) has proven to be practical in the preparation of a calcium-free solution containing plasma proteins, ions and organic constituents present in physiological concentrations. This ion-exchange resin was used many years ago for the collection of unadulterated whole blood prior to storage by blood transfusion services[155] and was abandoned because of its adverse effects on platelets and other coagulation factors.[14]

After addition of known amounts of $CaCl_2$ to previously decalcified plasma, coagulation occurred promptly even with [Ca] levels below 10% of the physiological range. Addition of decalcified blood and plasma to syringes prefilled with crystalline $CaCl_2$ and heparin (*cf.* p.35) provided data from which a possible Ca^{++} difference in serum and plasma could be derived. Our data (*cf.* p.39) are in agreement with data from the literature[33, 144] indicating that this difference is insignificant ($p > 0.1$). The demonstration of an insignificant ($p > 0.1$) difference between Ca^{++}_P and Ca^{++}_{WB} (*cf.* p.41) suggests that red blood cells do not interfere with the Ca^{++} measurement. The preservative present in commercially available heparin solutions appears to have no effect. Therefore, Ca^{++} measurements can be done in whole blood samples drawn for blood gas analysis. However, the time required to reach reproducible electrode potential readings was markedly prolonged. The reason for this is not immediately clear. In addition, storage of whole blood samples at ambient room temperature may provoke pH changes by changes in pCO_2. This problem decreases the practicality of the method and therefore, routine Ca^{++} measurements were done in plasma. Changes in pH have not been a problem in plasma samples, provided the syringes used for collection are well sealed.

In view of the absence of *in vitro* effects by heparin we anticipated no *in vivo* effects. Indeed, Ca^{++}_P values measured before and after systemic heparinization in patients prior to cardiopulmonary bypass were not significantly different (*cf.* p.43).

Therefore, Ca^{++} can best be measured in plasma prepared from heparinized blood samples collected for blood gas analysis.

CHAPTER IV

FACTORS AFFECTING PLASMA IONIZED CALCIUM

Changes in metabolism and homeostatic control can produce significant alterations in plasma composition. In this chapter we will present data on the effect of some of these metabolic factors on Ca^{++}_P .

A. pH TITRATION CURVES

The experimental data from McLean and Hastings[81] have suggested that fluctuations in Ca^{++}_S concentrations secondary to a moderate change in pH are small. On the basis of theoretical considerations these authors have predicted an inverse proportionality between Ca^{++}_P and pH, where $\Delta \log Ca^{++}_S / \Delta pH = -0.36$. The discrepancy between predicted and measured changes in Ca^{++}_S as affected by pH was in part explained by the limited sensitivity of the frog heart method. In addition, some question was raised as to the validity of the predicted value.

Recent data[37, 41, 53, 85] have demonstrated an appreciable effect of pH on calcium ionization throughout the physiological pH range. In the pH span of 7.20 to 7.60, Moore[85] found a $\Delta \log Ca^{++}_S / \Delta pH$ ratio of approximately -0.40 while a ratio of -0.50 was reported by others.[101, 107, 136]

1. *In vitro.* Normal adult volunteers.

Venous blood was collected from ten male and ten female adult volunteers in syringes the dead spaces of which had been prefilled with a heparin solution (*cf.* p.23). Ca^{++}_P values were measured at actual venous pH and also at two additional pH values obtained by tonometry of whole blood (*cf.* p.40).

As expected, an inverse relationship between Ca^{++}_P and pH was found. This relationship, shown in figure 14, may be described by the following regression equation:

$$\log Ca^{++}_P = 5.7163 - 0.6315\ pH \qquad (r = 0.789)$$

According to figure 14, the ratio $\Delta \log Ca^{++} / \Delta pH = -0.63$.

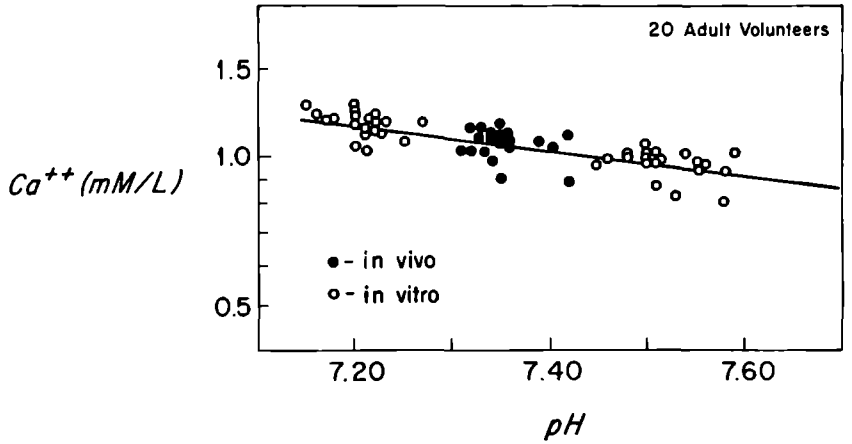

Fig. 14 — *In vitro* pH titration curve. Ca^{++}_P values were measured at actual pH in venous blood obtained from 20 normal adult volunteers and two different pH values obtained by tonometry. The solid line represents the regression equation $\log Ca^{++}_P = 5.7163 - 0.6315$ pH ($r = 0.789$)

2. *In vivo.* Patients undergoing open heart surgery.

In order to determine whether the *in vitro* pH effects applies *in vivo,* heparinized arterial blood samples were taken from ten cardiac surgical patients following different levels of ventilation during anesthesia in preparation for open heart surgery. Body temperature was 37° C.

In each patient, the first, or control sample was taken at a time when manually controlled ventilation was maintained at a level providing approximately normal minute ventilation as shown by repeated measurement of pH and pCO_2. The F_IO_2 was 0.40. Next, F_IO_2 was changed to 1.0 and a positive end-expiratory airway pressure[30] of 5 cm H_2O applied during a 4 minute period of apnea. At this point the second blood sample was taken. The F_IO_2 was decreased to 0.40 and manual hyperventilation continued for 20 minutes (V_T = 300 ml, f = 30 per minute) following which the third sample was taken. The infusion of different intravenous solutions was kept at a minimal rate (25 ml per hour). In each sample, Ca^{++}_P values were measured at the *in vivo* pH and at two additional pH values obtained by tonometry of whole blood (*cf.* p.40). Thus in each patient Ca^{++}_P values were determined at three *in vivo* and 6 *in vitro* pH values. The inverse relationship between

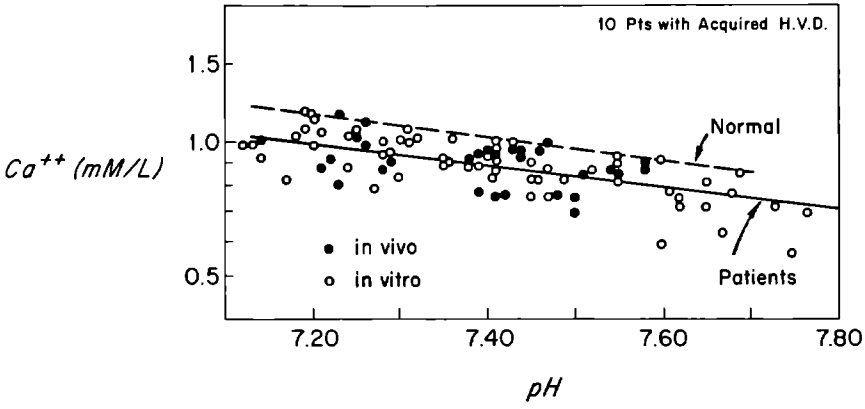

Fig. 15 — *In vivo* pH titration curves. In 10 patients with acquired heart valvular disease, Ca_P^{++} values were measured at different pH values produced either by a change in manually controlled ventilation or tonometry. Blood samples were drawn following induction of anesthesia using morphine in combination with an oxygen-nitrous oxide mixture. The solid line represents the regression equation: $\log Ca_P^{++} = 4.5404 - 0.4922$ pH ($r = 0.770$); the dotted line represents the regression equation graphically shown in fig. 14.

Ca_P^{++} and pH, illustrated in figure 15, is described by the following regression equation:

$$\log Ca_P^{++} = 4.5404 - 0.4922 \text{ pH } (r = 0.770)$$

The solid line (fig. 15) represents the regression equation for the patients and the dotted line the regression equation for normal adult volunteers (fig. 14). The differences in Ca_P^{++} at iso-pH values were statistically significant ($p < 0.01$). The ratio $\Delta \log Ca_P^{++} / \Delta$ pH was -0.49, similar to the value found by others.[101, 107] This value is higher than predicted by McLean and Hastings.[83]

B. TEMPERATURE

There are two possible effects of temperature on Ca_P^{++} : first, a direct, or primary one, produced by a change in the physicochemical equilibrium; second, an indirect or secondary one, produced via temperature induced changes in pH. The effect of increase in temperature *per se* would tend to increase the binding of calcium to protein[78] while decrease of pH (produced by increase in temperature) will tend to

decrease binding of calcium (figs. 14 and 15). We have studied the effect of temperature changes on Ca^{++} measurement by maintaining the electrode at 25° or 37° C.

$CaCl_2$ was added in varying amounts to 30 plasma samples of volunteers prepared as described previously (*cf.* p.35) to yield Ca^{++}_P values encompassing the range encountered clinically. The actual venous pH values at 37° C ranged from 7.36 to 7.47. Ca^{++}_P values were measured with the electrode maintained at 25° and 37° C (fig. 16). The relationship between Ca^{++}_{25} and Ca^{++}_{37} may be expressed by the following regression equation:

$$Ca^{++}_{25} = 0.02 + 0.9863\ Ca^{++}_{37} \qquad (r = 0.98)$$

These results indicate that in the range of 25° to 37° C the two described opposing effects of changes in temperature are approximately of the same magnitude.

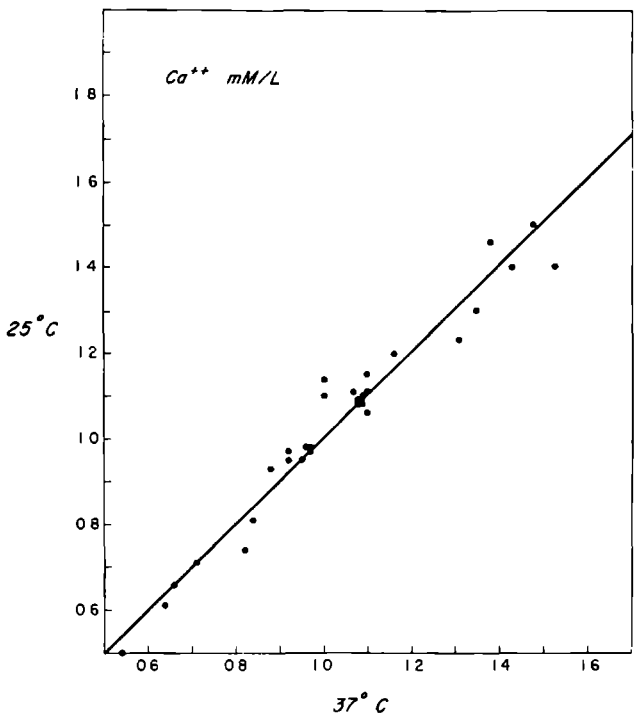

Fig. 16 — Effect of temperature on Ca^{++}_P. Measurements were made at 25° and 37°C. The relationship between Ca^{++}_{25} and Ca^{++}_{37} may be expressed by the regression equation: $Ca^{++}_{25} = 0.02 + 0.9863\ Ca^{++}_{37}$. The solid line represents the line of perfect agreement.

C. A.-V. DIFFERENCE

All reports in the literature have described Ca^{++}_P values measured in serum or plasma prepared from venous blood. Although it is likely that systemic differences are small, we wanted to exclude these as possible sources of error.

Ca^{++}_P values were measured in plasma samples prepared from blood taken simultaneously from a central venous pressure catheter, position of the tip being verified by X-ray, and an indwelling radial artery cannula in patients recovering from surgery. Twenty four sample pairs were analyzed. Some were taken after intravenous administration of a 1 gram bolus of $CaCl_2$. Care was taken that the $CaCl_2$ injection was not made via the venous line from which sampling was contemplated. The pH values of all venous and arterial specimens were equalized by tonometry (*cf.* p.40). According to figure 17 and table 4 there was close agreement between Ca^{++}_P values measured in central venous and in arterial blood after tonometry to identical pH ($r = 0.977$).

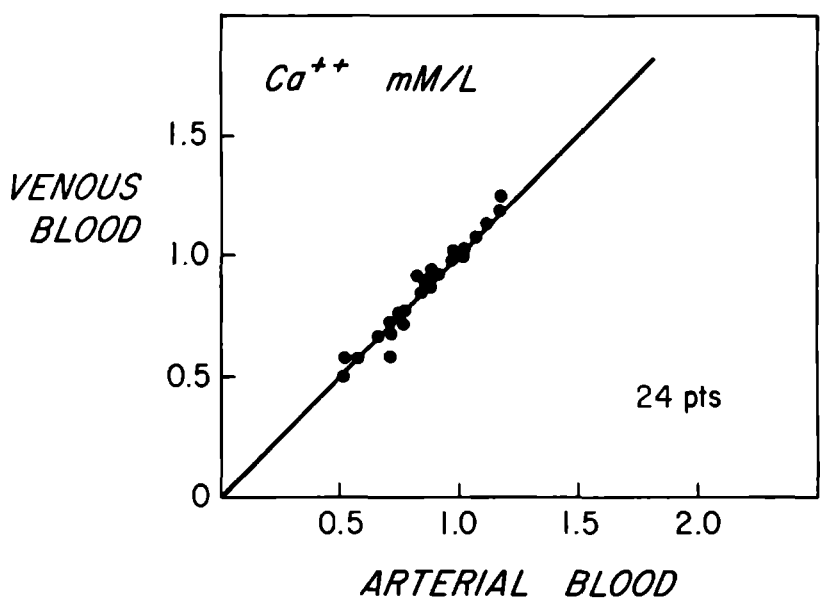

Fig. 17 — Comparison between Ca^{++}_P measured in arterial and central venous blood. Blood samples were drawn simultaneously from central venous pressure catheters and radial artery cannulae in patients recovering from major abdominal surgery. pH values were equalized by tonometry. Agreement was close ($r = 0.977$).

	N	IONIZED CALCIUM (mM/L) (MEAN ± S.D.)	TOTAL CALCIUM (mM/L) (MEAN ± S.D.)
ARTERIO-VENOUS DIFFERENCE	24	+ 0.014 ± 0.02	+ 0.024 ± 0.024
P		N.S.	N.S.

Table 4. Arterio-venous difference in [Ca] and Ca^{++}_P in patients recovering from major abdominal or vascular surgery.

D. HEMOLYSIS

If calcium binds to hemoglobin, a significant decrease in Ca^{++}_P might be expected to occur when free hemoglobin is present due to severe hemolysis. To test this hypothesis, Ca^{++} values were determined in plasma and hemolysates prepared from venous blood obtained from ten normal adult volunteers and from arterial blood obtained from ten patients in recovery room. Hemolysates were prepared by centrifuging the heparinized blood samples at 3000 *g* for 15 minutes, removing the supernatant plasma and freezing the red cells at −19° C for three hours. Ca^{++} values were measured in the plasma samples and in the hemolysates. As shown in table 5 the amounts of calcium present in the

	N	Ca^{++}_P (mM/L)*	$Ca^{++}_{HEMOLYSATE}$ (mM/L)*	Hb IN HEMOLYSATE (G/100 ML) *
SURGICAL PATIENTS IN RECOVERY ROOM	10	0.81 ± 0.17	BELOW DETECTION LIMIT OF ELECTRODE	25.7 ± 3.12
P		< 0.01		
NORMAL ADULT VOLUNTEERS	32	1.01 ± 0.07	BELOW DETECTION LIMIT OF ELECTRODE	28.4 ± 2.92

* M ± S.D.

Table 5. Ca^{++}_P in plasma and in hemolysates (see text) prepared from blood drawn from normal adult volunteers or from patients recovering from surgery. Although extracellular Ca^{++}_P was significantly different, no difference was in intracellular Ca^{++}_P could be measured, due to limited electrode sensitivity at low Ca^{++}_P values.

hemolysates was below the detection limit of the electrode (0.05 mM/L, *cf.* p.26). This is in agreement with data from the literature.[21] Thus, the effect of changes in extracellular Ca^{++} on intracellular concentrations could not be documented. To examine the effect of binding of calcium by hemoglobin, known volumes of hemolysate were added to previously decalcified and heparinized plasma, prepared from blood taken from a normal adult volunteer, in which [Ca] had been made 1.50 mM/L (Ca^{++}_P = 0.70 mM/L). Addition of the same volumes of a 150 mM NaCl solution to aliquots of this sample yielded a correction factor for the volume changes. Next, adjustment of pH was achieved by tonometry (*cf.* p.40).

According to figure 18, plasma hemoglobin levels up to 10 g/100 ml do not significantly alter Ca^{++}_P. A significant decrease of Ca^{++}_P however, is seen at a plasma hemoglobin level of 14 g/100 ml. The described effect of hemoglobin is expected to be of similar or smaller magnitude at higher Ca^{++}_P levels.

E. MAGNESIUM

Among the divalent cations, plasma magnesium may cause interference with the Ca^{++}_P measurement. This is due to an electrode response to magnesium ion activity (*cf.* p.26).

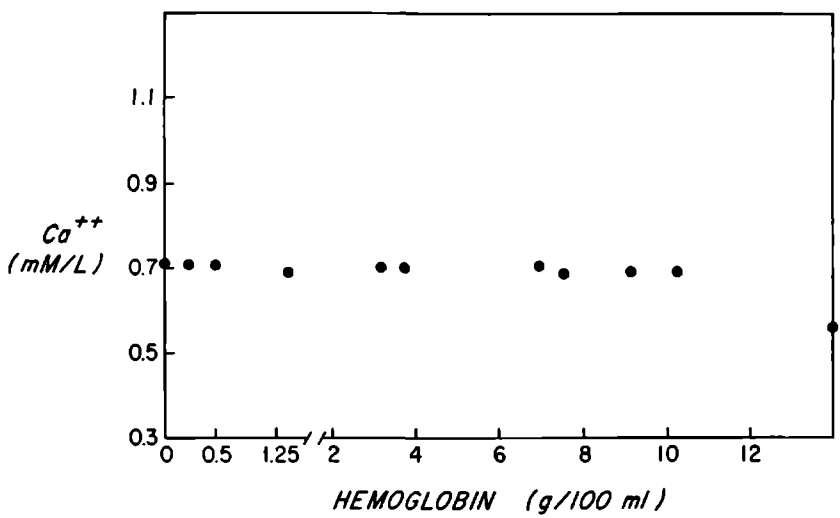

Fig. 18 — Effect of hemolysis. Increasing amounts of hemolysates were added to plasma of known Ca^{++}_P concentration. A hemoglobin level of up to 10 g/100 ml did not significantly lower Ca^{++}_P.

In order to determine the [Mg] level at which a significant error in Ca^{++}_P measurement occurs, whole blood drawn from an adult volunteer was passed over an ion-exchange resin (*cf.* p.35). [Ca], Ca^{++}_P and [Mg] levels were below detectable limits after treatment. Next, the heparinized blood sample was divided into two portions. $CaCl_2$ was added to make [Ca] 1 or 2 mM/L (Ca^{++}_P = 0.68 or 1.33 mM/L, respectively); to each of these two Ca^{++} concentrations we added $MgCl_2$ to achieve [Mg] concentrations of 2, 4, 9, 12 or 14 mEq/L. Evaporation of these solutions prior to addition of plasma eliminated potential errors due to volume changes (*cf.* p.35). Aqueous $CaCl_2$ solutions were prepared using standard solutions containing 0.5 or 1.0 mM/L $CaCl_2$, respectively and 150 mM/L NaCl to which additional $CaCl_2$ was added to yield similar Ca^{++} values as measured in plasma. Prior to addition of crystalline $CaCl_2$ pH values of the solutions were adjusted to 7.35 with triethanolamine. Known increasing amounts of $MgCl_2$ were added to these solutions as described above. According to figure 19, a [Mg] level as high as 9 mEq/L has no appreciable effect on Ca^{++} measurement, both in aqueous and in protein containing solutions; higher [Mg] concentrations can produce significant errors. At a [Mg] level of 14 mEq/L, the error was approximately 8%. With these high levels of [Mg] the electrode response time was markedly prolonged. The reason for this is not immediately clear.

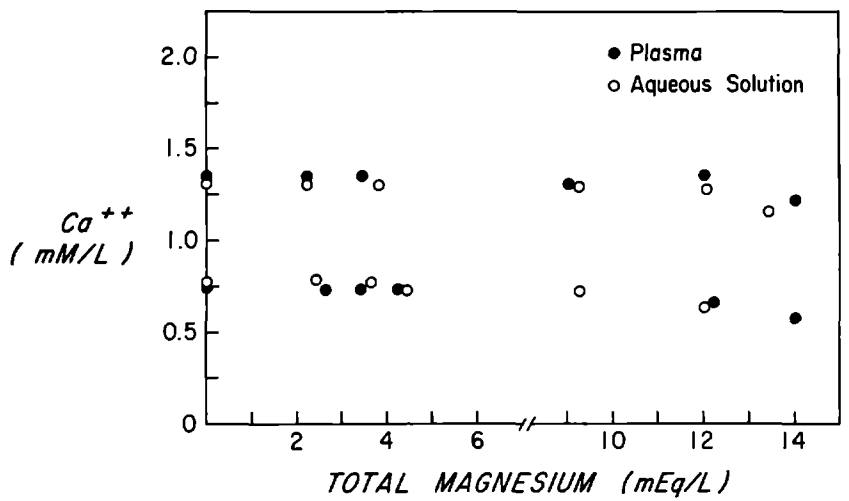

Fig. 19 — Effect of magnesium concentration on Ca^{++} in plasma and aqueous solution. A significant depression of apparent Ca^{++} was observed at [Mg] levels of above 9 mEq/L.

F. ANIONS

Complexation of calcium to anions, particularly if their concentration is abnormally high, is expected to occur in circulatory failure (due to lactate accumulation), renal failure (due to retention of phosphate) or during rapid transfusion of citrated whole blood (due to the accumulation of citrate). The *in vitro* concentration of each of these anions at which a decrease of Ca^{++} occurs was investigated.

1. Lactate

We intended to prepare sodium lactate solutions of increasing concentration, and to add $CaCl_2$ to make final [Ca] = 0.5, 1.0 or 2.0 mM/L; however crystalline sodium lactate was not available. Calcium lactate solutions were, therefore, prepared, containing increasing amounts of that salt (ranging from 0.05 to 28 mM/L). These solutions were then passed over an ion-exchange resin (*cf.* p.35) so that sodium lactate solutions of increasing concentrations resulted; NaCl was added to make Na^+ 138 mEq/L, verified by flame photometry. These solutions

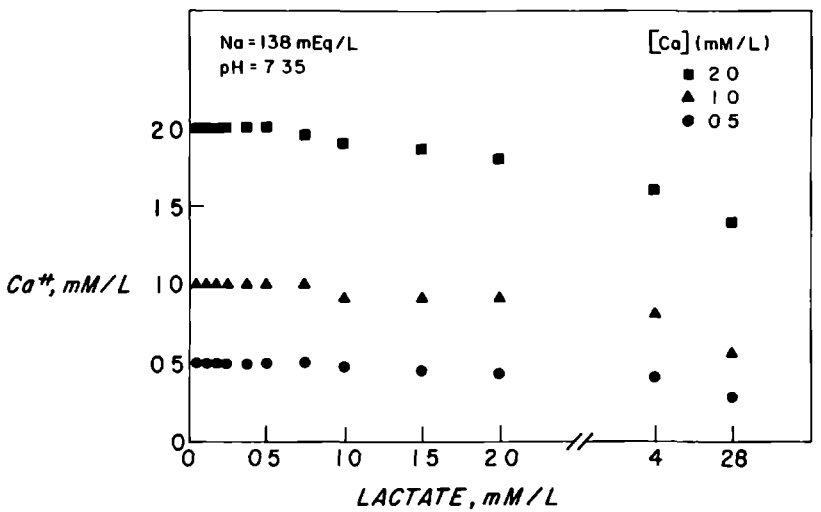

Fig. 20 — Effect of lactate on Ca^{++} in aqueous solutions. NaCl was added to lactate solutions of increasing concentration to make final Na^+ 138 mEq. $CaCl_2$ was then added to make [Ca] 0.5,1.0 or 2.0 mM/L. Below a lactate concentration of 0.8 mM/L, [Ca] and Ca^{++} were identical, indicating that no significant complexation had occurred. Above this lactate concentration a progressive increase in the difference between [Ca] and Ca^{++} was observed. At a lactate concentration of 28 mM/L, this difference was 0.73 mM/L at [Ca] = 2 mM/L.

were then added to crystalline $CaCl_2$ in 5 ml syringes to make [Ca] values 0.5, 1.0 or 2.0 mM/L (*cf.* p.35). The results are presented in figure 20. At lactate levels below 0.8 mM/L there was no significant difference between [Ca] and Ca^{++}; *i.e.,* significant complexation had not occurred. However at higher lactate concentrations a progressive increase of the difference between [Ca] and Ca^{++} was apparent; at a lactate concentration of 4 mM/L (commonly observed in a state of moderate metabolic acidosis), this difference was 0.41 mM/L ([Ca] = 2 mM/L) and at a lactate concentration of 28 mM/L (present in Ringer's lactate solution), this difference was 0.73 mM/L ([Ca] = 2 mM/L).

2. Phosphate

Sodium dibasic phosphate solutions were prepared with NaCl added to a final sodium concentration of 150 mEq/L. P_I levels were verified according to the method described (*cf.* p.27) and Na^+ levels, by flame photometry. The pH ranged from 7.47 to 7.54. Each phosphate solution was added to crystalline $CaCl_2$ in 5 ml syringes to make [Ca] 0.5, 1.0 or 2.0 mM/L, according to the technique described previously (*cf.* p.35). According to figure 21, a progressive increase in the difference between [Ca] and Ca^{++} was observed. At a P_I level of 3 mM/L this difference was 1.35 mM/L ([Ca] = 2 mM/L).

3. Citrate

Trisodium citrate solutions of increasing concentration were prepared and NaCl added to a final sodium concentration of 150 mEq/L. Each of these citrate solutions was then added to crystalline $CaCl_2$ in 5 ml syringes to make [Ca] 0.5, 1 or 2 mM/L, according to the technique described previously (*cf.* p.35). The results are presented in figure 22. There was a progressive increase in the difference between [Ca] and Ca^{++} values with increasing citrate concentration. At a citrate level of 10 mM/L (commonly found during rapid blood transfusion[16]) this difference was 0.75 mM/L ([Ca] = 2 mM/L), indicating significant binding at that citrate concentration.

G. PROTEINS

The chief calcium-binding substance in plasma is protein. Data from the literature[85] have indicated a significant difference of calcium-binding

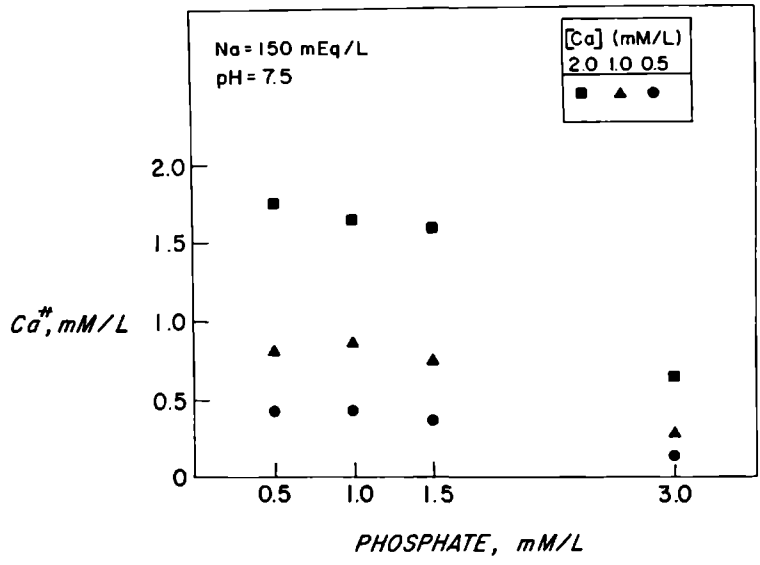

Fig. 21 — Effect of phosphate on Ca^{++} in aqueous solutions. Sodium dibasic phosphate solutions of increasing concentrations were prepared and NaCl added to make Na^{+} 150 mEq/L. $CaCl_2$ was added to make [Ca] 0.5, 1.0 and 2.0 mM/L. A progressive increase in the difference between [Ca] and Ca^{++} was observed.

properties between albumin and globulin; albumin has been shown to have higher affinity than globulin.

The pH dependent interaction between protein and calcium ions will be discussed (*cf.* p. 59). The clinical observation of adverse hemodynamic effects following the infusion of plasma protein fractions[10] prompted our *in vitro* investigation of the calcium-binding properties of commercial protein solutions.

Commercial salt-poor albumin (25%) solution was dialyzed with 10 times its volume of a buffered salt solution (Na^{+} = 135 mEq/L, Cl^{-} = 105 mEq/L, bicarbonate = 25 mEq/L, pH = 6.2) using an ultrafiltration cell* (membrane XM-50), pressurized with oxygen at 1.6 atm. Following this procedure the protein solution was concentrated and then passed over an ion-exchange resin (*cf.* p. 35) to remove the calcium. The concentrated protein solution was then diluted

* Amicon, Lexington, Massachusetts

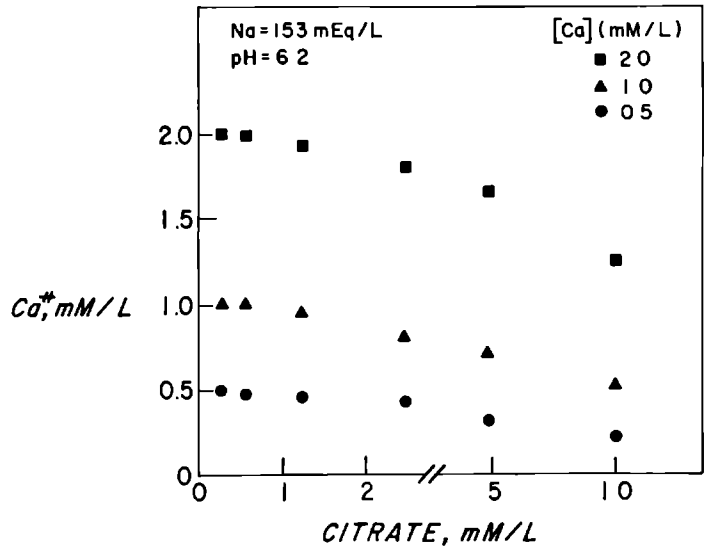

Fig. 22 — Effect of citrate on Ca^{++} in aqueous solutions. Trisodium citrate solutions of increasing concentration were prepared and NaCl added to make Na^{+} 153 mEq/L. $CaCl_2$ was added to make [Ca] 0.5,1.0 or 2.0 mM/L. Above a citrate concentration of 0.2 mM/L there was a progressive increase of the difference between [Ca] and Ca^{++}.

with the buffered salt solution to make solutions with T.P. values ranging from 1.5 to 8 g/100 ml as determined by refractometry, and pH adjusted to 7.35 by tonometry (*cf.* p.40). Each solution was then added to crystalline $CaCl_2$ in 5 ml syringes to make [Ca] 2 mM/L according to the technique previously described (*cf.* p.35). The results are presented in figure 23. In the T.P. range 3 to 7.5 g/100 ml no significant difference in Ca^{++} was observed in spite of decrease in binding capacity; below 3 g/100 ml a significant increase in Ca^{++} and above 7.5 g/100 ml, a decrease in Ca^{++} was observed, as expected. In order to compare the protein-calcium interactions observed in a commercial protein solution with those observed in a plasma protein solution prepared from blood drawn from a normal adult volunteer, peripheral venous blood was passed over an ion-exchange resin using the collection system shown in figure 8. The red cells were separated from the plasma by centrifugation and the calcium-free plasma dialyzed and concentrated using an ultrafiltration cell as described (*cf.* p.56). After addition of 50 I.U. heparin per 5 ml plasma, protein solutions ranging in T.P. from 1 to 8 g/100 ml were prepared using the balanced salt

solution described above. Each solution was then added to crystalline $CaCl_2$ in 5 ml syringes to make [Ca] 2 mM/L, according to the technique described previously (*cf.* p.35). The results are presented in figure 23. There was a progressive increase in Ca^{++} with decrease in protein concentration over the entire range studied. Comparison of these results with those observed with commercial protein solutions (salt-poor albumin) reveals that the latter display a significantly higher affinity for calcium. At a T.P. of 5.7 g/100 ml this difference was approximately 0 25 mM/L ([Ca] = 2 mM/L).

H. DISCUSSION

Several normally occurring plasma constituents may alter Ca^{++}_P either by direct interaction or indirectly by their effect on homeostatic control mechanisms. Therefore, in the evaluation of Ca^{++}_P values definition of some of these factors is required. Some factors exert their effects on Ca^{++}_P measurement itself, for example, unphysiologically high [Mg] levels leading to inappropriately low Ca^{++}_P values Other factors may be of importance in the explanation of a discrepancy be-

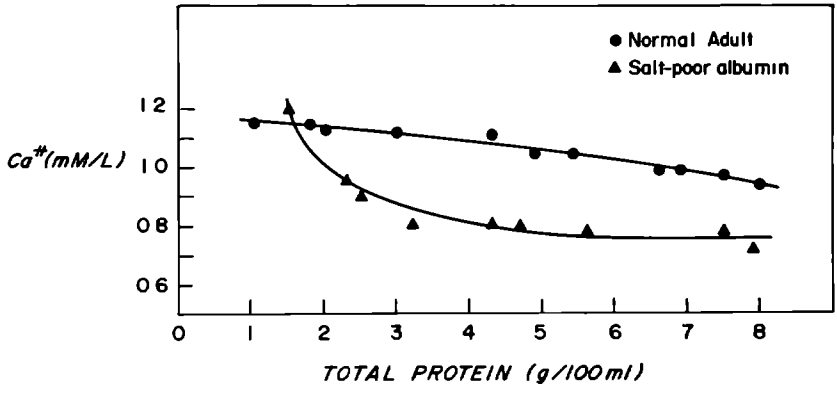

Fig. 23 — Effect of total protein on Ca^{++}. Blood obtained from a normal adult volunteer was decalcified using an ion-exchange resin (*cf* p.35) and the plasma was washed and concentrated using an ultrafiltration cell, pressurized with oxygen (1.6 atm). Protein solutions were then prepared by adding a buffered salt solution (see text) to yield total protein values ranging from 1 to 8 g/100 ml $CaCl_2$ was added to each solution to make [Ca] 2 mM/L; pH adjustment was made by tonometry (*cf.* p.40). As expected, Ca^{++} increased with decreasing T. P. A commercial protein solution (salt-poor albumin) was treated in the same fashion. The latter displayed different calcium-binding characteristics at a T. P. of 5.7 g/100 ml Ca^{++} in native protein solution was 0 25 mM/L higher than the commercial protein solution. The titration curve of salt-poor albumin with Ca^{++} was not linear

tween Ca^{++}_P values predicted from the McLean-Hastings nomogram[83] and those directly measured, for example, high citrate levels leading to low Ca^{++}_P values secondary to chelation.[143] Empirical formulas that form the basis for the McLean-Hastings nomogram do not take these factors into account.

The effect of pH on Ca^{++}_P measurement in aqueous solutions containing a background of 150 mM NaCl has been discussed (*cf.* p.25). Below pH 5.5 the electrode no longer measures exclusively Ca^{++} activity but H^+ activity as well. Calculations[85] have indicated that the dissociation of each of the complexes physiologically present in plasma should not vary with changes of pH within the physiological range. This has been confirmed experimentally by Moore.[85] Therefore, we may assume that effects of changes of pH on Ca^{++} are largely accounted for by changes in calcium-binding of proteins. These changes are completely reversible; for example, we have found that Ca^{++} in plasma samples before exposure to air (during which CO_2 loss occurs with concomitant change in pH) were identical with those after tonometry with 8% CO_2/92% O_2 used to restore the original pH. This may suggest a competition of H^+ and Ca^{++} ions for protein binding sites. We have determined the effect of changes in pH on Ca^{++}_P by tonometry of plasma and whole blood with 8% CO_2/92% O_2 or air on the assumption that pH, not pCO_2 is the factor determining calcium binding to proteins. This is supported by the findings of Gupta,[41] Loken[78] and Lindgärde.[75] We have observed that the effects of changes in pH *in vitro* and *in vivo* were of comparable magnitude (figs. 14 and 15). It is of interest to compare our *in vivo* data to those presented by Lindgärde[75] who noted less of an effect of pH on Ca^{++} in venous blood taken from adult volunteers following active hyperventilation. Perhaps homeostatic control mechanisms are impaired in anesthetized man.

The effect of temperature changes on Ca^{++}_P we observe is the sum of direct and indirect effects. McLean and Hastings[81] were unable to investigate the effect of temperature in the span 25 - 37° C as the frog heart loses its sensitivity toward Ca^{++} above 30° C. Therefore, these authors have reported Ca^{++}_S values at 22 - 25° C, suggesting that the temperature correction for 37° C would probably be small. Loken *et al.*[78] have demonstrated with ultracentrifugation of serum at different temperatures that protein binding of Ca^{++} at 37° C is greater than at 25° C, the difference being on the order of 5%. The indirect effect of

changes in pH induced by temperature changes will have an opposite effect on binding of calcium. Hansen and Theodorsen[43] and Moore[106] have compared Ca^{++}_S values at different temperatures using the calcium electrode maintained at 25° and at 37° C and have found that the difference in Ca^{++}_S secondary to changes in temperature in the span 25 - 37° C are on the order of 0.02 mM/L. Our results are in agreement. Therefore, we may conclude that for practical purposes correction for body temperature is not necessary.

Ca^{++}_P values measured in venous and arterial blood have been generally assumed to be identical but there are no data to support this assumption. Our findings have demonstrated that significant systemic differences do not occur in the physiological Ca^{++}_P range. If pH is the only important factor we may assume that Ca^{++}_P values measured in peripheral venous blood are identical with those measured in arterial blood.

Hemolysis can occur whenever high vacuum is present in the syringe or glass tube employed for collection of blood or when a small bore needle is used. We usually observe some hemolysis during extracorporeal perfusion in patients undergoing open heart surgery. We wanted to exclude this factor as a possible source of error in Ca^{++}_P measurement anticipating that Ca^{++}_P might bind to hemoglobin if Ca^{++} levels within red cells are low. Data from the literature[21] have indicated that intracellular Ca^{++} levels are on the order of 10^{-7} M. In accord with these data we have found that Ca^{++} levels in hemolysates prepared from blood taken from volunteers and from patients recovering from surgery are below the detection limit of the electrode (table 5). From figure 18 we may conclude that the amount of free plasma hemoglobin during blood collection or encountered in patients undergoing operation does not represent a source of significant error.

The presence of a high plasma [Mg] level in association with a low Ca^{++}_P observed in a patient in respiratory failure prompted investigation of the effect of [Mg] on Ca^{++}_P measurement. Our *in vitro* experiments indicate (*cf.* p.52) that [Mg] concentrations below 9 mEq/L do not interfere with Ca^{++} measurement. Depression of apparent Ca^{++} was observed with [Mg] levels above 12 mEq/L.

Several anions known to appear in significant concentrations during certain clinical situations can bind calcium in substantial quantities. This complexation may be of sufficient magnitude to account for some of

the low Ca^{++}_P levels encountered in patients in "low-flow" state (*vide infra,* p.77). Lactate and phosphate have their effect by ion-pair formation while citrate is a chelating agent.[143] Further investigation is needed to correlate plasma lactate, phosphate and citrate concentrations with Ca^{++}_P .

Although the adverse hemodynamic effect of commercial protein solutions has been shown[10] to relate to vasodilator substances present in certain commercially available plasma protein fractions, it was first thought that lowering of Ca^{++}_P might be a contributing factor. This does not appear to be the case, however, since hemodynamic changes were observed after intravenous infusion of as little as 25 ml. The difference in calcium binding between normal plasma and commercial proteins (fig. 23) observed *in vitro* warrants investigation of its clinical significance; it is possible that changes in protein patterns in critically ill patients can occur and that these may be partially responsible for the low Ca^{++}_P values observed (*cf.* p.86).

CHAPTER V

CRITICAL CARE EXPERIENCE WITH IONIZED CALCIUM MEASUREMENTS

It has been generally stated that calcium salts are useful in the correction of poor hemodynamic function and it has been assumed that the calcium ion has a positive inotropic action. Although this effect has been demonstrated in animal heart muscle preparations[28, 81, 110] there are no *in vivo* data available to demonstrate its mechanism of action in man.

Since we have found that post-operative Ca^{++}_P values measured in patients without obvious hemodynamic changes were generally lower than those found in normal adult volunteers, we were interested in defining a range of Ca^{++}_P values in man following operation. This Ca^{++}_P could serve as an end-point for calcium replacement therapy and could be useful in the interpretation of abnormally low Ca^{++}_P values, characteristically encountered in patients during "low-flow" states (*vide infra,* p.77).

In this chapter we will present our findings in patients prior to and following operation; we will discuss the hemodynamic response to intravenously administered calcium chloride at different rates in patients with and without ganglionic blockade; and present data on patients in "low-flow" states.

A. PLASMA IONIZED CALCIUM LEVELS IN PATIENTS UNDERGOING ELECTIVE SURGERY

Ca^{++}_P values for normal adult volunteers have been presented (*cf.* p.29). However, "normal" values found in adult volunteers do not necessarily apply to patients during recovery from surgery. Pre- as well as intraoperative factors are capable of significantly altering Ca^{++}_P patterns. Well known examples are immobilization[7], resulting in elevation of Ca^{++}_P, rapid infusion of citrated whole blood[14-16, 60, 61] resulting in a decrease of Ca^{++}_P; and changes in hydrogen ion activity, resulting in either a decrease or increase of Ca^{++}_P (figs. 14 and 15).

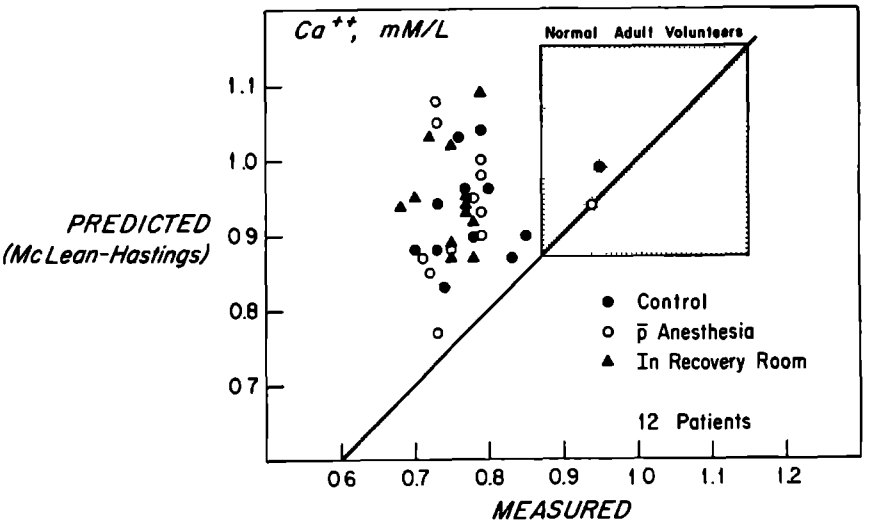

Fig. 24 — Relationship between measured Ca^{++}_{P} values in 12 patients undergoing elective major abdominal surgery and those predicted from the McLean-Hastings nomogram. Blood samples were drawn immediately prior to and 15 minutes following induction of anesthesia and within 3 hours following admission to the recovery room. Ca^{++}_{P} values were lower than those found in normal adult volunteers and lower than predicted. The shaded area indicates the 95% confidence limits of Ca^{++}_{P} values in normal volunteers.

In order to establish a range of Ca^{++}_{P} values in surgical patients in whom there were no obvious hemodynamic changes intra- or postoperatively, measurements were made in blood samples taken from 12 patients (age range 21-64 years) admitted to the hospital for elective major abdominal operations. Of these patients, 7 underwent cholecystectomy, 2 of which were followed by common bile duct exploration; 3 patients underwent abdominal hysterectomy; and 2, subtotal gastrectomy. These patients did not have any systemic disorders known to affect calcium metabolism; preoperative values for blood urea nitrogen were below 25 mg/100 ml and for serum potassium, above 3.8 mEq/L. All patients received preoperative medication consisting of a barbiturate, an opiate and atropine, 1 hour prior to the time of scheduled operation. Peripheral venous blood samples were taken immediately prior to and 15 minutes following induction of anesthesia and within 3 hours following admission to the recovery room. Agents used in the course of anesthesia included thiopental, morphine, enflurane and non-depolarizing muscle relaxants. The duration of the operation

in each patient was less than 3 hours; plasma protein fractions or citrated whole blood had not been administered for 3 hours prior to the blood sampling. The pulse rate was below 100 beats per minute and urine output was at least 40 ml per hour. Calculated mean arterial blood pressures before and after induction of anesthesia and at termination of operation were above 75 torr, without the use of vasopressors.

Ca^{++}_P values measured prior to and following induction of anesthesia and following termination of the operation were lower than predicted (fig. 24) and lower than those found in normal adult volunteers (fig. 25 and table 6). These differences were statistically significant ($p<0.01$).

B. CHANGES IN PLASMA IONIZED CALCIUM LEVELS FOLLOWING INTRAVENOUS INFUSION OF $CaCl_2$

The generally accepted belief that the calcium ion has positive inotropic action in man has probably contributed to the popularity of calcium salts as suitable agents in the correction of poor hemodynamic function. Calcium gluconate and calcium chloride are the most commonly used calcium salts. It has been said that calcium gluconate is safer than calcium chloride since cardiac arrhythmias are less frequently associated with administration of the former. A possible explanation

	Patients with Elective Surgery (N = 12)			Normal adult Volunteers
	Control	15 min. after Anesthesia	Recovery Room	(N = 32)
Ca^{++} (mM/L)*	0.78 ± 0.06	0.77 ± 0.06	0.75 ± 0.03	1.01 ± 0.07
P	< 0.01	< 0.01	< 0.01	
[Ca] (mM/L)*	2.12 ± 0.15	2.06 ± 0.14	2.04 ± 0.12	2.34 ± 0.197
P	< 0.01	< 0.01	< 0.01	
P_I (mM/L)*	0.94 ± 0.25	0.91 ± 0.28	0.97 ± 0.19	0.95 ± 0.13
P	N.S.	N.S.	N.S.	
T.P. (G/100 ML)*	6.99 ± 0.51	6.77 ± 0.51	6.50 ± 0.61	7.31 ± 0.51
P	< 0.05	< 0.02	< 0.01	
pH *	7.35 ± 0.05	7.37 ± 0.06	7.32 ± 0.04	7.37 ± 0.05
P	N.S.	N.S.	N.S.	

* Mean ± S.D.

Table 6. Values of Ca^{++}_P, [Ca], P_I, T. P. and pH measured in plasma prepared from blood obtained from 12 patients admitted for elective abdominal surgery (see text). The p values are relative to normal adult volunteers.

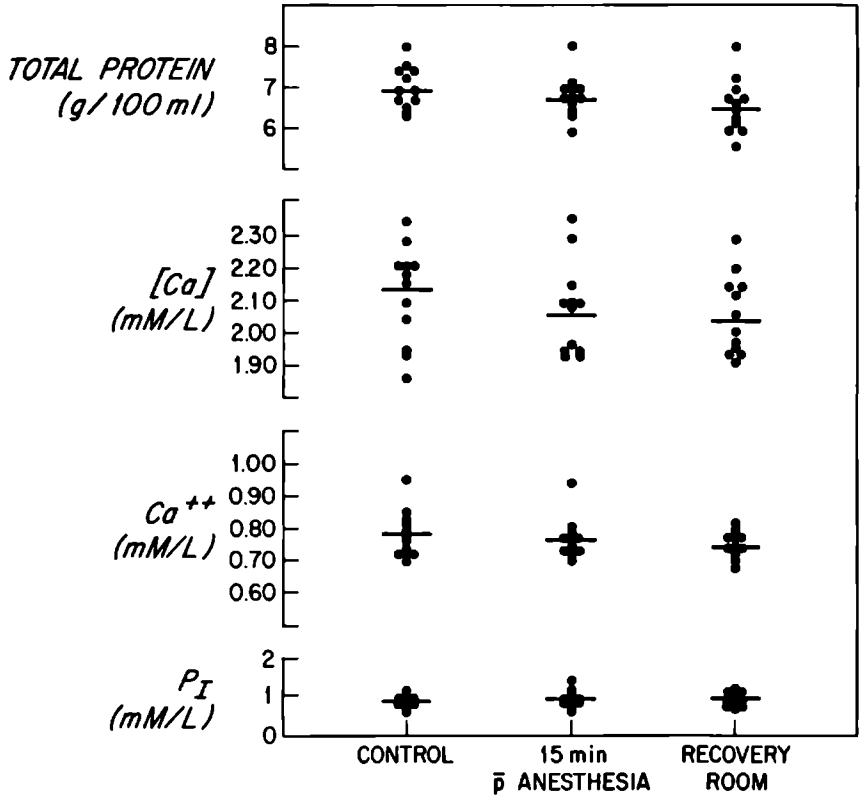

Fig. 25 — Ca^{++}_P , [Ca], P_I and T. P. values measured in the patients presented in fig. 24. There was no significant change in mean Ca^{++}_P with induction of anesthesia or following the operation.

for this observation is that commercial ampoules of $CaCl_2$ are more concentrated than those containing calcium gluconate; furthermore, the chloride salt is more highly dissociated in solution.[143] It might be expected, therefore, that lesser quantities of the chloride salt are required to raise Ca^{++}_P .

We have been interested in Ca^{++}_P changes that appear following intravenous infusion of $CaCl_2$ at different rates. The $CaCl_2$ solutions were infused with a constant infusion pump through an indwelling central venous pressure catheter the tip of which had been demonstrated to be lying centrally by X-ray examination. We have followed the Ca^{++}_P patterns in 5 patients recovering from major abdominal surgery in whom low Ca^{++}_P values were associated with poor

hemodynamic function. We arbitrarily chose a total dose of 2 grams $CaCl_2$ which was then infused at different rates. Thus, at 16 mg per minute, the infusion required a total of 2 hours; at 50 mg per minute 40 minutes; and at 100 mg per minute, 20 minutes. Our choice for the lowest rate was based on the clinical practice of adding 2 grams $CaCl_2$ to an intravenous infusion of either isoproterenol (0 4 mg) or epinephrine (1 mg) where dilution is usually achieved in 250 ml of 5% dextrose in water and an average of 1 ml is administered per

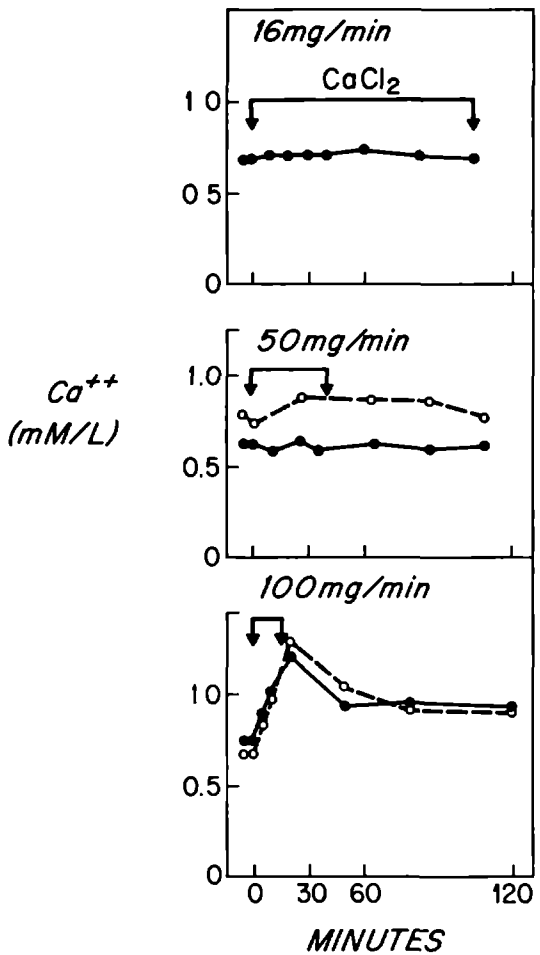

Fig. 26 — Effect of administration rate on Ca^{++}_{P} A total of 2 grams $CaCl_2$ was given intravenously at different rates to 5 postoperative patients with low initial Ca^{++}_{P} and poor hemodynamic function. Rates below 100 mg per minute were ineffective in raising Ca^{++}_{P} Therefore a slow intravenous infusion of $CaCl_2$ is probably not useful for elevation of Ca^{++}_{P}.

minute (*i.e.*, 8 mg $CaCl_2$ per minute). Thus our lowest dose was twice the quantity infused clinically. The results are presented in figure 26. There was no significant rise in Ca^{++}_P when $CaCl_2$ was administered at a rate of 16 mg per minute in one patient. At a rate of 50 mg per minute there was no rise in one patient and a small rise in the other. Thus, slow intravenous infusion of $CaCl_2$, running over several hours is probably of little value if an elevation of Ca^{++}_P is to be achieved. At 100 mg per minute, however, Ca^{++}_P did rise significantly in two patients and remained well above control levels for several hours.

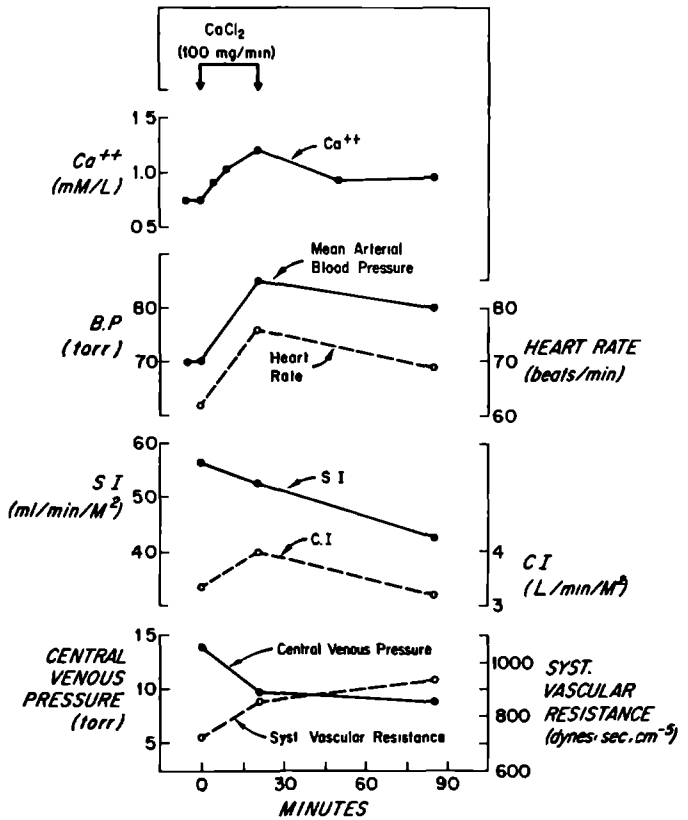

Fig. 27 — Hemodynamic effects of $CaCl_2$ administered intravenously at a rate of 100 mg per minute in a patient recovering from major vascular surgery. The rise in mean arterial blood pressure was due to increases in heart rate and systemic vascular resistance. Note that this was the only patient in which heart rate increased markedly in response to $CaCl_2$ administration. The significance of changes in central venous pressure remains to be determined.

C. HEMODYNAMIC RESPONSE TO RAPID INTRAVENOUS ADMINISTRATION OF $CaCl_2$ IN THE POSTOPERATIVE PERIOD

The intravenous administration of $CaCl_2$ by constant infusion might be expected to produce gradual changes in Ca^{++}_P and hemodynamic function. In figure 27 those changes are illustrated in a patient recovering from major vascular surgery who was given 2 grams of $CaCl_2$ at a rate of 100 mg per minute in an attempt to correct poor hemodynamic function. It is clinical practice, however, to administer $CaCl_2$ at a rate of 200 mg per minute or above to patients in whom the arterial blood pressure is low.

In 19 patients (age range: 34 to 79 years) recovering from major abdominal or vascular surgery, measurements were made just prior to and 15 minutes following rapid intravenous administration of $CaCl_2$ (figs. 28-30, table 7). Surgery had been completed at least 24 hours prior to the study and whole blood or blood products had not been given for at least 3 hours. These patients required pharmacologic cir-

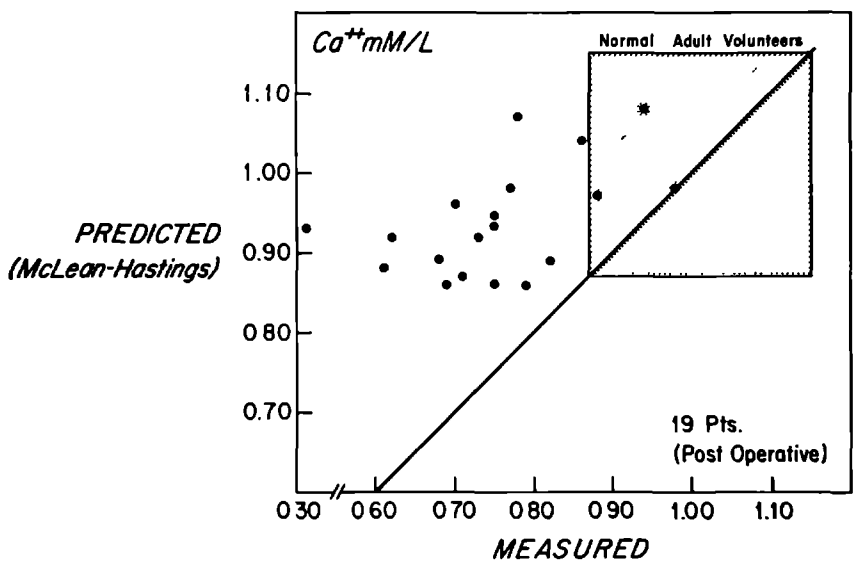

Fig. 28 — Relationship between Ca^{++}_P measured and predicted from the McLean-Hastings nomogram. In 19 patients recovering from major abdominal or vascular surgery Ca^{++}_P was lower than in normal adult volunteers and lower than predicted. These patients received $CaCl_2$ in an attempt to improve hemodynamic function (figs. 29 and 30). The shaded area indicates the 95% confidence limits of Ca^{++}_P values in normal volunteers.

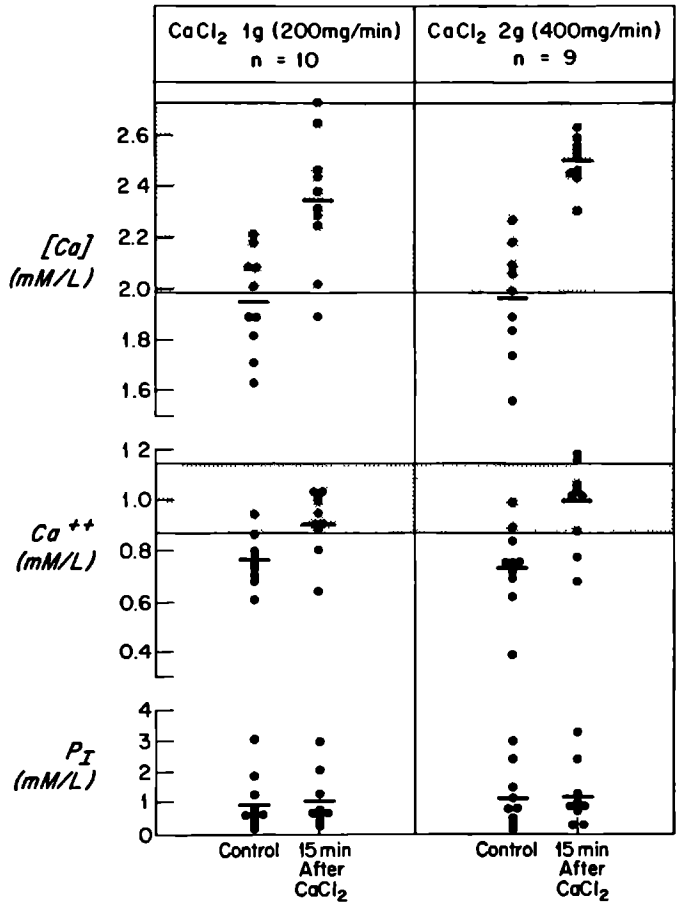

Fig. 29 — Effect of administration of $CaCl_2$ at different rates on [Ca], Ca^{++}_P and P_I. Postoperative patients received 1 g $CaCl_2$ (200 mg per minute, n = 10) or 2 g (400 mg per minute, n = 9) and blood samples were drawn immediately prior to and 15 minutes following $CaCl_2$ administration. No effect was observed on P_I. Both [Ca] and Ca^{++}_P increased significantly following $CaCl_2$ injection but were independent of the total dose. The shaded areas indicate the 95% confidence limits of Ca^{++}_P values in normal volunteers.

culatory support to maintain adequate arterial blood pressure or urinary output.

Ten patients (group I) received 1 gram $CaCl_2$ (200 mg per minute) and nine patients (group II), 2 grams (400 mg per minute). Prior to $CaCl_2$ administration, Ca^{++}_P values in both groups were significantly ($p<0.01$) *lower* than those found in normal adult volunteers (fig. 29)

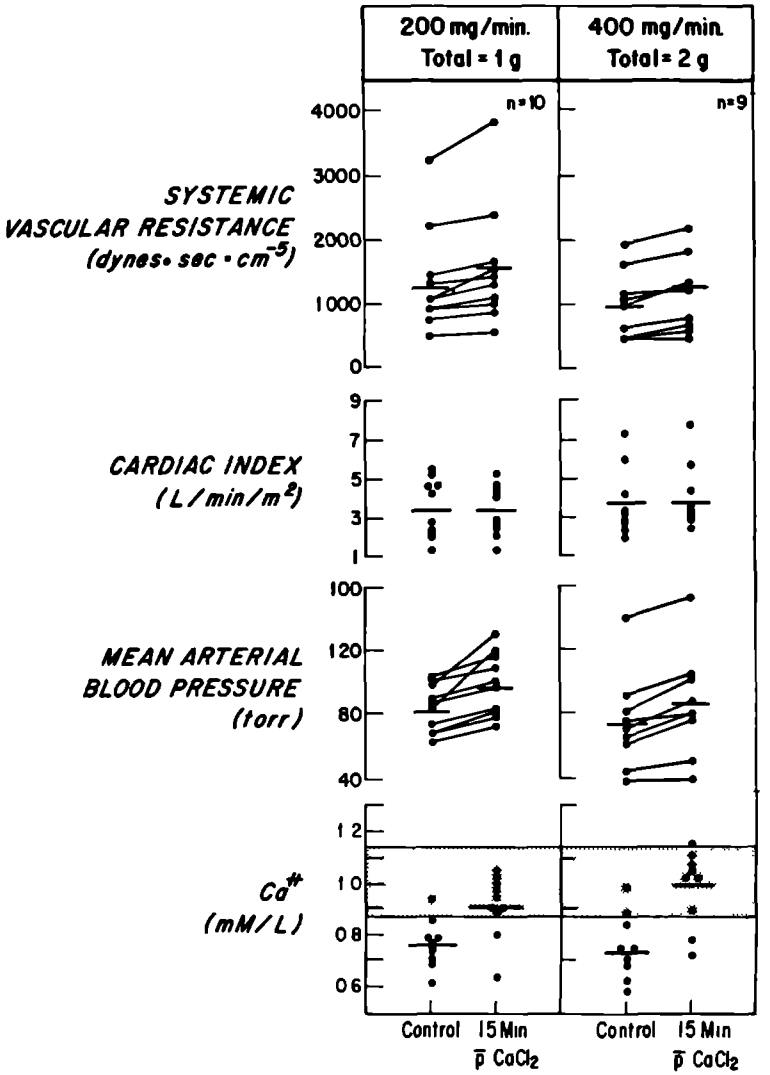

Fig. 30 — Hemodynamic effect of $CaCl_2$ administration in postoperative patients. Following the intravenous injection of $CaCl_2$, Ca^{++}_P rose in all patients; simultaneously, significant increases in mean arterial blood pressure and systemic vascular resistance were observed while cardiac index did not change. Thus, the rise in mean arterial blood pressure was due to elevation of *systemic vascular resistance* and appeared independent of the total dose. The shaded area indicates the 95% confidence limits of Ca^{++}_P values in normal volunteers.

Total Dose of $CaCl_2$	Time after Injection (minutes)	Per Cent Changes From Control					
		Ca^{++}_P *	Mean Art. Blood Pr. *	Cardiac Index *	Stroke Volume *	Systemic Vasc. Resistance *	Heart Rate *
1 gram 200 mg/min. n = 10	15 P	+ 24.3 ± 5.0 < 0.01	+ 18.0 ± 4.0 < 0.01	+ 3.7 ± 3.6 n.s.	+ 4.4 ± 5.3 n.s.	+ 12.0 ± 3.0 < 0.01	+ 2.4 ± 1.6 n.s.
2 grams 400 mg/min. n = 9	15 P	+ 36.7 ± 8.7 < 0.01	+ 14.6 ± 3.4 < 0.01	+ 2.3 ± 2.7 n.s.	+ 4.9 ± 4.2 n.s.	+ 14.1 ± 3.1 < 0.01	0 ± 3.0 n.s.

* Mean ± S.E.

Table 7. Changes in Ca^{++}_P and in hemodynamic parameters following i.v. administration of $CaCl_2$ to patients recovering from major abdominal or vascular surgery.

and lower than predicted from the McLean-Hastings nomogram (fig. 28), but *similar* to Ca^{++}_P measured in patients undergoing elective abdominal surgery (*cf.* p.63, fig. 25). In all patients, Ca^{++}_P and [Ca] values rose significantly ($p<0.01$) following intravenous administration of $CaCl_2$ (1 or 2 grams). Mean Ca^{++}_P rose from 0.74 to 0.92 mM/L (group I) and from 0.71 to 0.97 mM/L (group II) as shown in figure 29. There was no statistically significant ($p>0.1$) difference between Ca^{++}_P values measured in group I and those in group II 15 minutes following administration of $CaCl_2$, although values prior to $CaCl_2$ administration in both groups were similar. Thus 1 gram $CaCl_2$ (200 mg per minute) was as effective as 2 grams (400 mg per minute). Simultaneously, there was a significant ($p<0.01$) rise in mean arterial blood pressure (mean change: 12.1 torr) in all patients, both 1 and 2 gram doses yielding similar changes in blood pressure, while there was no significant ($p>0.1$) change in cardiac output, stroke volume or heart rate. The rise in mean arterial blood pressure, therefore, was exclusively due to a significant ($p<0.01$) increase in *systemic vascular resistance* (mean increase: 12% [group I] and 14.1% [group II]).

D. HEMODYNAMIC RESPONSE TO RAPID INTRAVENOUS ADMINISTRATION OF $CaCl_2$ DURING HYPOTENSIVE ANESTHESIA

In recent years there has been an active search for suitable techniques to reduce the need for whole blood transfusion during operations. This search has primarily been stimulated by the demonstrated red cell lesions produced during blood storage[12] and the potential pulmonary complications that may follow embolization of particulate matter secondary to massive transfusion[22, 54, 92].

Jennings *et al.*[55] have shown in 50 orthopedic surgical patients undergoing total hip replacement that deliberate hypotensive anesthesia resulted in a significant reduction (approximately 50%) in the amount of whole blood transfused. This deliberate hypotension was induced by the intravenous administration of pentolinium tartrate after satisfactory anesthesia and relaxation had been achieved with halothane and *d*-tubocurarine.

To avoid postoperative bleeding it is desirable that arterial blood pressure be restored prior to wound closure to levels measured before administration of pentolinium tartrate. Antagonism of the hypotensive effects of this agent is generally achieved by changes in posture and the use of vasoconstrictor agents[68, 158]. These drugs do not restore vasomotor control and may, in fact, restore blood pressure at the expense of tissue perfusion[158]. An undesirable side effect may be the increase in myocardial work secondary to an increased left ventricular afterload.

Data obtained from experiments in dogs[152] have suggested that $CaCl_2$ might be effective in restoring low arterial blood pressure resulting from intravenous administration of chlorpromazine. In man, a return of arterial blood pressure to levels measured before the induction of hypotensive anesthesia has been observed to occur following rapid intravenous administration of $CaCl_2$ at termination of the operation.[26]

These considerations provided us with an opportunity to examine the hemodynamic response to intravenously administered $CaCl_2$ in patients under deliberate hypotensive anesthesia using a combination of halothane and pentolinium tartrate.

In 7 patients undergoing total hip replacement, measurements of hemodynamic parameters and Ca_P^{++} were made immediately prior to, 3 to 5 minutes and 25 to 30 minutes following the rapid intravenous injection of $CaCl_2$ (1 gram during 30 seconds) via a central venous

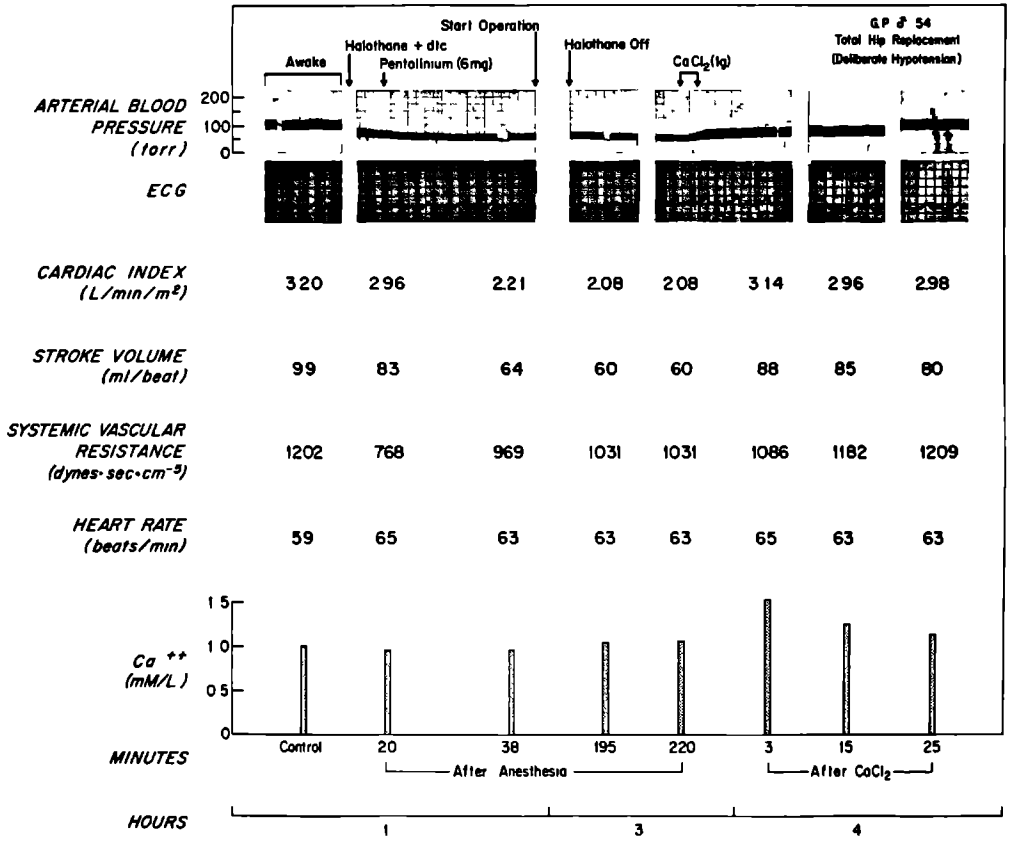

Fig. 31 — Hemodynamic effects of hypotensive anesthesia and their reversal using $CaCl_2$ in a patient undergoing total hip replacement. Following induction of anesthesia, the decrease in stroke volume was partially compensated by an increase in heart rate; after administration of pentolinium tartrate a significant reduction in stroke volume was observed. Simultaneously no appreciable change in Ca^{++}_P occurred. At the end of the operation, $CaCl_2$ (1 gram) was given over 30 seconds via the central venous pressure catheter, halothane being discontinued 20 minutes prior to that time. A significant increase in arterial blood pressure resulted from increase in stroke volume (3 minutes) while increase in systemic vascular resistance contributed to the arterial blood pressure rise at 25 minutes.

pressure catheter. In one of these patients measurements were also made immediately prior to and 15 minutes following induction of anesthesia, and immediately prior to and 3 minutes following the administration of pentolinium tartrate (figs. 31 and 32). In all patients, body temperature decreased in the first hour to approximately 34° C due to the use of laminar airflow in the operating room.

The results are presented in figures 31-34 and in table 8. Prior to injection of $CaCl_2$, Ca^{++}_P values were significantly ($p<0.01$) lower than those measured in normal adult volunteers; mean Ca^{++}_P was 0.87 mM/L. Significant increases in Ca^{++}_P and [Ca] were observed 3 minutes ($p<0.01$) and 25-30 minutes ($p<0.01$) following the intravenous administration of $CaCl_2$. No significant ($p>0.1$) changes were noted in P_I, pH or T.P. values. Simultaneously, there were significant

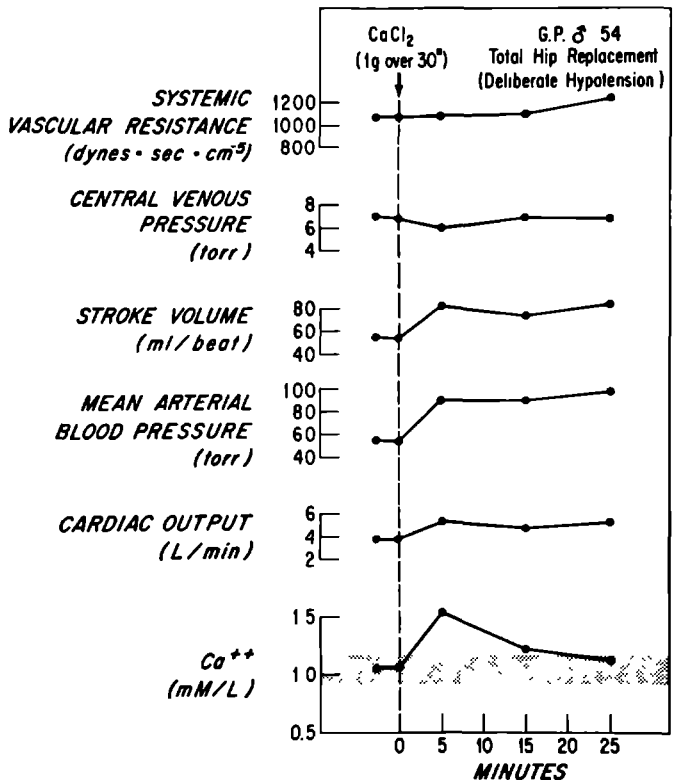

Fig. 32 — Hemodynamic parameters shown in patient presented in fig. 31. The shaded area indicates the 95% confidence limits of Ca^{++}_P values in normal volunteers.

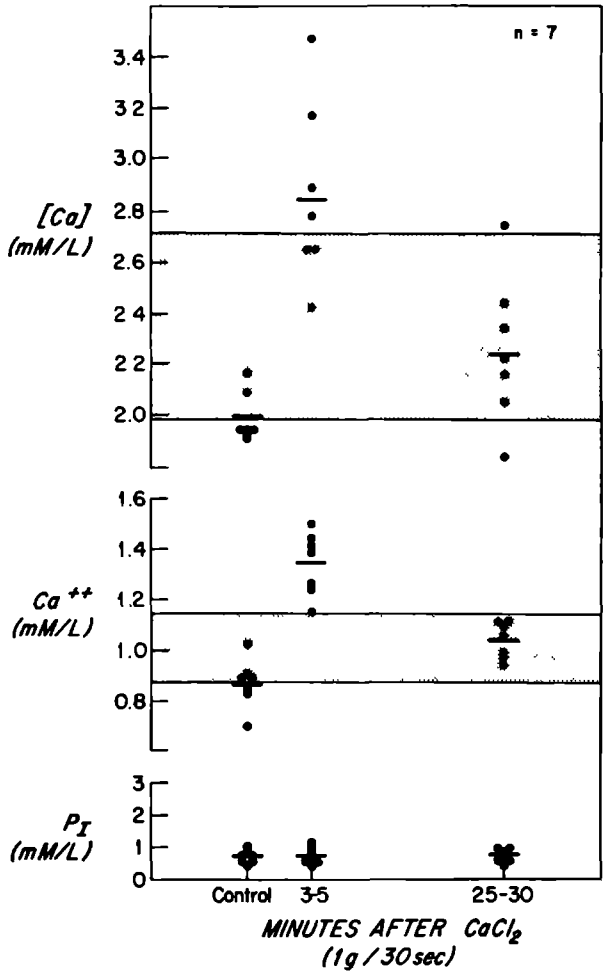

Fig. 33 — Effect of rapid intravenous injection of $CaCl_2$ (1 gram in 30 seconds) to 7 patients under delibrate hypotensive anesthesia for total hip replacement. Control samples were drawn immediately prior to the $CaCl_2$ injection. No change was observed in P_I while significant changes were noted in [Ca] and Ca^{++}. The shaded areas indicate the 95% confidence limits of Ca^{++} values in normal volunteers.

increases in mean arterial blood pressure ($p<0.01$), stroke volume ($p<0.01$) and cardiac output ($p<0.01$) 3 minutes following i.v. $CaCl_2$. No significant changes were recorded in systemic vascular resistance, heart rate or central venous pressure. The rise in mean arterial

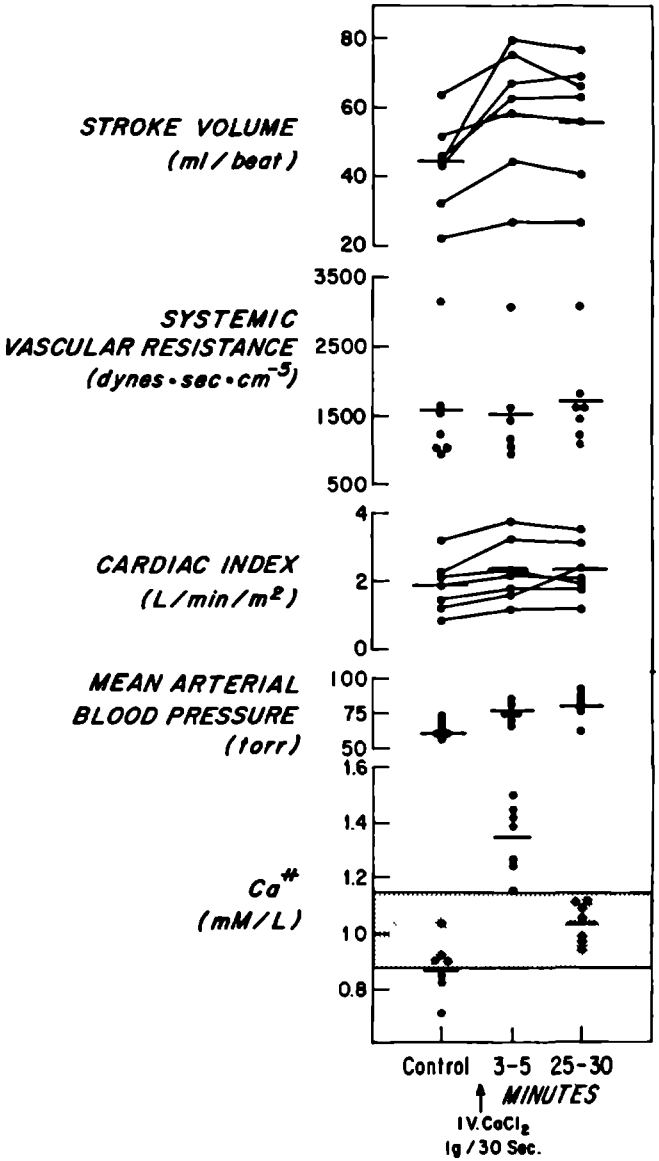

Fig. 34 — Hemodynamic effects of rapid intravenous injection of $CaCl_2$ (1 gram over 30 seconds) in 7 patients under deliberate hypotensive anesthesia for total hip replacement. Significant increases were observed in mean arterial blood pressure, cardiac index and stroke volume (3-5 minutes) while an increase in systemic vascular resistance contributed to the blood pressure rise after 25-30 minutes. Thus the immediate effect of $CaCl_2$ administration was an increase in mean arterial blood pressure resulting from increase in *stroke volume*. The shaded area indicates the 95% confidence limits of Ca^{++}_P values in normal volunteers.

Time after Injection of $CaCl_2$ (min)	N	Per Cent Changes From Control					
		Ca^{++}_P *	Mean art. Blood Pr. *	Cardiac Index *	Stroke Volume *	Systemic Vasc. Resistance *	Heart Rate *
3 - 5	7	+ 53.1 ± 10.8	+ 22.4 ± 3.1	+ 25.2 ± 6.3	+ 23.4 ± 8.2	+ 1.0 ± 4.6	0 ± 1.0
	P	< 0.01	< 0.01	< 0.01	< 0.01	N.S.	N.S.
25 - 30	7	+ 19.9 ± 5.5	+ 27.0 ± 6.1	+ 19.3 ± 8.3	+ 28.3 ± 9.4	+ 14.1 ± 6.8	0 ± 1.4
	P	< 0.01	< 0.01	< 0.01	< 0.01	< 0.05	N.S.

* Mean ± S.E.

Table 8. Changes in Ca^{++}_P and in hemodynamic parameters following i.v. administration of $CaCl_2$ to patients under deliberate hypotensive anesthesia.

blood pressure, therefore, was due to an increase in *stroke volume.* These values remained elevated 25 to 30 minutes following the $CaCl_2$ administration, at which time an increase in systemic vascular resistance contributed to the elevation of mean arterial blood pressure.

E. DYNAMICS OF IONIZED CALCIUM CONTROL DURING "LOW-FLOW" STATES IN CRITICALLY ILL MAN

Abnormally low Ca^{++}_P values are among the various abnormal biochemical parameters we have found associated with the presence of a "low-flow" state, defined as an acute situation in which hemodynamic function (*i.e.,* arterial blood pressure, cardiac output) is inadequate, resulting in poor tissue perfusion (pallor, cyanosis, or slow capillary refill at the extremities, poor urinary output) and requiring circulatory support (vasopressors).

These findings were made during routine monitoring of Ca^{++}_P levels in 8 such patients two of whom had non-surgical conditions and 6 of whom had undergone cardiac surgery for congenital or acquired heart disease (table 9). Only one of these patients survived. This severe hypocalcemia appeared in the absence of signs of increased neuromuscular irritability. In none of these patients was tetany present.

Age	Weight (kg)	Diagnosis	Arterial Blood				
			pH	Ca++ (mM/L)	[Ca] (mM/L)	P_I (mM/L)	T.P. g/100 ml
18 y	72.3	Gram negative septicemia	7.25	0.18	1.97	2.18	5.4
44 y	65.4	Systemic Intoxication	7.20	0.33	1.28	1.14	5.8
2 d	3.1	Atrial septostomy (T.G.V.)	7.05	0.21	1.48	2.47	5.8
2 d	2.9	Aortic coarctation repair	7.01	0.33	1.43	1.14	4.8
54 y	58.6	Aortic and mitral Valve Repl. (Rheumatic valvular disease)	7.44	0.45	2.76	0.98	7.9
18 mos	11.6	Repair Tetralogy of Fallot	7.46	0.24	1.54	1.31	5.2
9 mos	7.2	Repair Tot. Anom. Pulmonary venous drainage	7.38	0.53	1.89	1.62	5.7
2 d	3.2	Repair Tot. Anom. Pulmonary venous drainage	7.43	0.52	2.49	0.46	6.2

Table 9. Summary of data on patients in "low-flow" state. [Ca], $Ca^{\leftrightarrow}_{P}$, P_I and T.P. values are given at the lowest pH encountered.

Patient 1, an 18–year old female (fig. 35) was admitted in respiratory failure because of pneumonia, later developing gram–negative sepsis, requiring mechanical ventilation and pharmacological circulatory support.

Patient 2, a 44–year old male, was admitted with respiratory failure following systemic intoxication with isopropyl alcohol.

Patient 3 was a 2–day old newborn (fig. 36) in whom circulatory failure followed atrial septostomy for transposition of the great arteries.

Patient 4 was a 2-day old newborn who developed circulatory failure following repair of severe aortic coarctation.

Patient 5 was a 54–year old female in whom a "low flow" state developed following aortic and mitral valve replacement for rheumatic valvular disease.

Patient 6 was an 18–month old child (fig. 37) requiring circulatory support following repair of tetralogy of Fallot.

Patient 7 was a 9–month old child who developed circulatory failure following repair of partial anomalous pulmonary venous drainage.

Patient 8 was a 2–day old newborn (fig. 38) in whom a "low output" state developed following repair of total anomalous pulmonary venous drainage.

In four of these patients, circulatory insufficiency was associated with significant metabolic acidosis refractory to sodium bicarbonate admin-

istration. In the remainder acid-base balance was normal. There was no obvious relationship between degree of acidosis and Ca^{++}_P levels. Low Ca^{++}_P values were associated with low [Ca]; however, the decrease in Ca^{++}_P was disproportionally great. In only 2 patients were P_I values significantly elevated; in 3 patients they were normal; in the remaining patients they were abnormally low. T.P. values were below those found in adult volunteers in all patients but one. In this patient 300 ml salt-poor albumin had been administered during the 4 hours preceding blood sampling, resulting in a value of 7.9 g/100 ml.

In patient 1 (fig. 35) administration of 2 grams $CaCl_2$ over a 2 hour period did not result in restoration of normal Ca^{++}_P. Simultaneously, mean arterial blood pressure rose with each 1 gram dose of $CaCl_2$ but this rise was only transient. Pulmonary capillary wedge and central venous pressures showed a small decrease; the significance of these changes remains to be determined.

Patient 2 showed a quite similar response to intravenous $CaCl_2$. In both patients each 1 gram dose resulted in only a small increase in Ca^{++}_P. In view of the possible complications that may follow the rapid administration of $CaCl_2$[49-51], particularly cardiac arrhythmias, it was felt at that time that no more $CaCl_2$ was justified.

In view of the absence of cardiac arrhythmias in these two patients and of the small rise in Ca^{++}_P, we subsequently administered larger quantities of $CaCl_2$ to the other patients studied. In figures 36-38 this extraordinary $CaCl_2$ requirement is shown.

In patient 4 severe and intractable hypocalcemia and metabolic acidosis were present postoperatively. At autopsy, congenital absence of the parathyroid glands was noted.

Patient 6 (fig. 37) appeared to be refractory to several vasoactive agents (epinephrine, isoproterenol) and since Ca^{++}_P was low, it was thought that administration of as much $CaCl_2$ as was needed to restore Ca^{++}_P to normal might improve hemodynamic function. Although up to 3000 mg $CaCl_2$ had been given in a 24 hour period without significant increase in arterial blood pressure, administration of less than half that amount on day 3 resulted in a conspicuous rise of Ca^{++}_P (1.82 mM/L), that was lowered by partial exchange of whole blood with salt poor albumin. Similar intolerance to calcium replacement therapy was observed on day 13, following slow intravenous infusion of renal

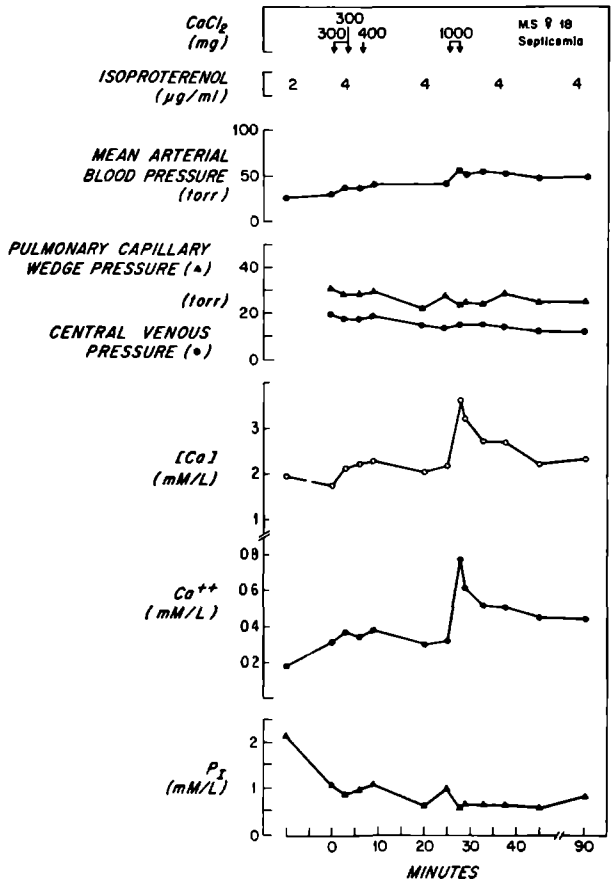

Fig. 35 — Effect of $CaCl_2$ administration in an 18-year old female admitted with respiratory failure due to viral pneumonia and septicemia. Following injection of each gram $CaCl_2$ a new plateau was reached, but a normal Ca^{++}_P value was not sustained. Although [Ca] was low, Ca^{++}_P was disproportionally low. Simultaneously, with each Ca^{++}_P plateau, a new arterial blood pressure level was reached, but not sustained after the second 1 gram dose. Slight decreases of pullmonary capillary wedge and central venous pressures were noted.

failure fluid* and peritoneal dialysis both solutions containing $CaCl_2$. The former contained 2 mM phosphate, the latter did not contain phosphate. This may in part explain the extraordinarily low P_I values observed.

In patient 8 (fig. 38) an interesting relationship between rate of isoproterenol infusion and Ca^{++}_P levels was found. Even large amounts

*Renal failure fluid (RFF), a solution containing essential amino acids, dextrose, and vitamins has been shown to improve survival from acute renal failure[1]

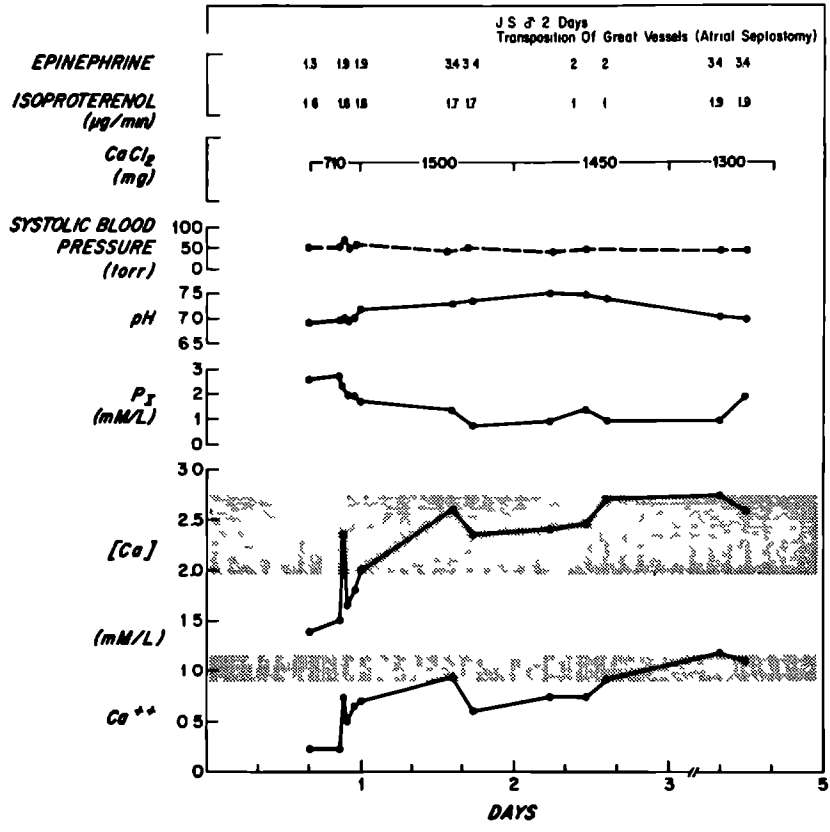

Fig 36 — Effect of $CaCl_2$ in a 2-day old infant following Blalock-Hanlon procedure for transposition of the great arteries Large amounts of $CaCl_2$ were given together with epinephrine and isoproterenol in an attempt to improve hemodynamic function A persistent metabolic acidosis (pH = 7 05, pCO_2 = 46 torr) was refractory to sodium bicarbonate At autopsy, infarction of the gastrointestinal tract reaching from the esophagus to the distal colon was found The shaded areas indicate the 95% confidence limits of Ca^{++}_P values in normal volunteers

of intravenously administered CaCl failed to restore normal Ca^{++}_P but increase of circulatory support from 2 9 to 4 8 μg isoproterenol per minute in conjunction with continued $CaCl_2$ administration promptly resulted in a return of Ca^{++}_P to within normal limits P_I values ranged from 0 56 to 0 73 mM/L

Acute hyperglycemia was observed in patients 5 and 8 (table 9) In patient 5, 25 units of crystalline zinc insulin given intravenously hourly were inadequate for restoration of normal blood sugar levels

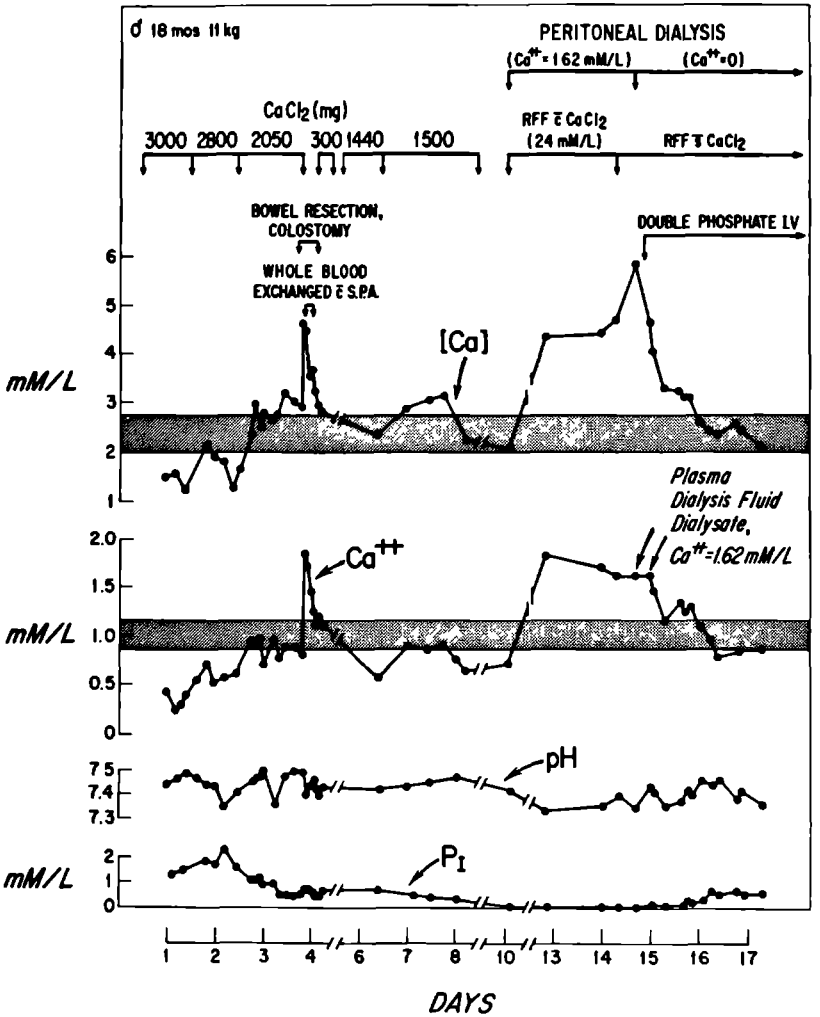

Fig. 37 — Effect of $CaCl_2$ administration at high doses to patient 6 (Table 9). Low initial [Ca] and Ca^{++}_P values were only corrected after very large doses $CaCl_2$. On day 3, conspicuous rises in [Ca] and Ca^{++}_P were observed, treated by partial exchange transfusion of whole blood with salt-poor albumin solution. Addition of $CaCl_2$ to peritoneal dialysis fluid and to renal failure fluid (see text) resulted in a sharp increase in Ca^{++}_P and [Ca] again on day 13-15 so that $CaCl_2$ was omitted from intravenous and peritoneal dialysis fluids. On day 15, plasma, peritoneal dialysis fluid and dialysate were in complete equilibrium at a Ca^{++}_P of 1.62 mM/L, slowly decreasing thereafter. Simultaneously P_I values decreased markedly requiring exogenous phosphate replacement. Even at high Ca^{++}_P levels hemodynamic function did not improve significantly. The shaded areas indicate the 95% confidence limits of Ca^{++}_P values in normal volunteers.

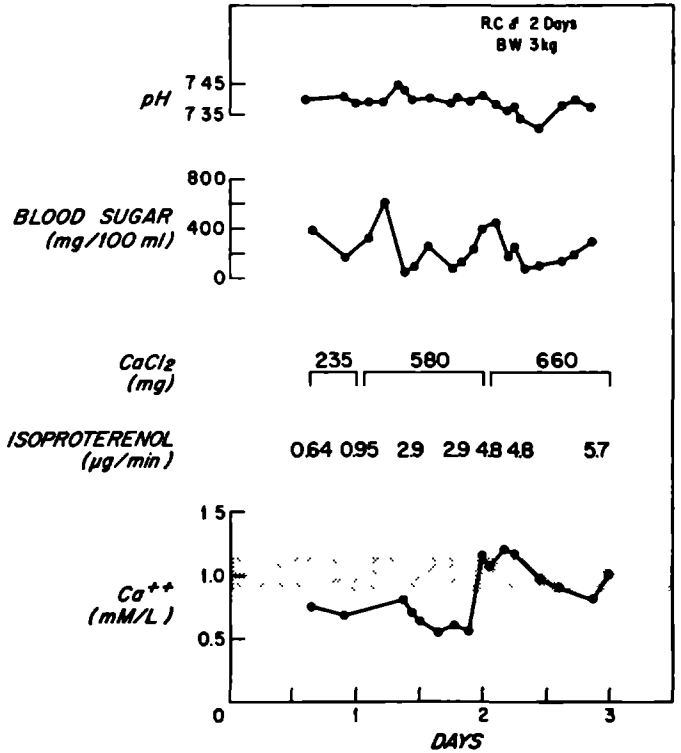

Fig. 38 — Effect of circulatory support (isoproterenol) on Ca^{++}_P . On day 1, 235 mg $CaCl_2$ given to this infant (postoperative, repair of total anomalous pulmonary venous drainage) were not sufficient to restore Ca^{++}_P to normal, nor were 580 mg, but increase in rate of isoproterenol administration in conjunction with continued $CaCl_2$ administration restored Ca^{++}_P to normal. No metabolic acidosis was present. Note that in the absence of glucose administration blood sugar levels were significantly elevated, particularly during the first two days. The shaded area indicates the 95% confidence limits of Ca^{++}_P in normal volunteers.

F. DISCUSSION

The relationship between hemodynamic function and Ca^{++}_P levels requires definition. This is of particular interest in critically ill patients in whom acute, pronounced and persistent alterations in calcium homeostasis have been found in association with changes in hemodynamic function.

Although a cause-effect relationship between Ca^{++}_P and hemodynamic performance has been documented in isolated animal heart muscle preparations[28, 110] and in animal preparations of the intact heart,[6, 28, 81] there are

few data that define this relationship in man.[14, 15, 70, 151] The isolated frog heart studies by McLean and Hastings have shown that in the physiological Ca^{++}_P range, a close correlation existed between extracellular Ca^{++}_P and amplitude of muscular contraction. Ebashi *et al.*[28] have defined the interaction between Ca^{++} and contractile proteins; Chidsey[21] suggested that the delivery of calcium to these proteins from intracellular stores is limited and dependent on extracellular concentration. An abnormality in calcium flux, therefore, might depress contractility of the mycardium.

Human studies relating serum calcium and hemodynamic function have been undertaken in neonates[151], children[18] and adults[15]. An investigation of the hemodynamic effects of decrease in Ca^{++}_P secondary to citrate infusion in anesthetized man has shown[15] that a lowering of mean arterial blood pressure was consistently associated with a decrease in stroke volume. In agreement with these findings are reports on hypocalcemic hypotension in a child[18] and on neonatal heart failure[151] caused by hypocalcemia. Conversely, Feinberg *et al.*[32] have found that rapid increase in extracellular Ca^{++}_P (produced by intravenous administration of calcium gluconate) in isolated perfused dog hearts produced an increase in the rate of left ventricular pressure rise and an increase in heart rate. Furthermore, there is evidence to suggest an effect of calcium on systemic vascular resistance, either by direct effect on vascular smooth muscle or indirectly, by enhancement of catecholamine release from the adrenal medulla as demonstrated *in vitro*[39].

The calcium selective electrode has proven useful in the documentation of acute changes in Ca^{++}_P concentrations observed in critically ill patients. We have measured Ca^{++}_P values below 0.50 mM/L in these patients without overt electrocardiographic changes, a level shown by McLean and Hastings to be incompatible with frog heart action. This illustrates the limitation of extrapolation of data obtained from animal experiments to man.

For appropriate interpretation of abnormal Ca^{++}_P values there is a need for establishing a range of normal. We have, therefore, measured Ca^{++}_P in normal adult volunteers (*cf.* p.29). In addition, we have measured Ca^{++}_P levels in surgical patients before and after induction of anesthesia and following termination of an elective major abdominal operation (fig. 25). In the absence of demonstrable hemodynamic changes (calculated mean arterial blood pressures ranging from 75 to 96 torr) Ca^{++}_P values measured prior to and following induction of

anesthesia were similar but they were lower than those found in normal adult volunteers. Thus *low* Ca^{++}_P *does not necessarily occur secondary to anesthesia* but may be present *prior* to anesthesia and operation. Appreciation of these findings is important in the evaluation of Ca^{++}_P values measured in other patients studied. Simultaneously, T.P. and [Ca] were lower while P_I was not significantly different. In the face of lower protein concentration and therefore less binding capacity, Ca^{++}_P might be expected to be higher than in normal adult volunteers. It is possible that the fasting state is a factor contributing to the low Ca^{++}_P values observed. Preoperative medication may influence Ca^{++}_P . The degree to which anions participate in calcium binding and the possibility of abnormal protein patterns require further study.

It has been generally recommended that calcium salts be administered slowly, primarily in view of potential complications (cardiac arrhythmias) that may follow their administration[49-51]. On the basis of physiological considerations administration of $CaCl_2$ by constant infusion may be preferable. Our data, however, suggest that in the absence of impaired renal function administration of $CaCl_2$ at rates below 100 mg per minute (fig. 26) is of little value for the elevation of Ca^{++}_P .

In the presence of renal failure, Ca^{++}_P values as high as 1.82 mM/L have been encountered when slow $CaCl_2$ administration was continued over prolonged periods of time (fig. 37). In these circumstances it was difficult to return Ca^{++}_P to normal. In our experience exchange transfusion with salt-poor albumin was more rapidly effective than peritoneal dialysis (fig. 37).

In the correction of of inappropriate hemodynamic function 2 grams of $CaCl_2$ might be expected to be more effective than 1 gram, given over the same period of time. Our data, however (figs. 29 and 30) indicate that this is not the case. Increases in mean arterial blood pressure recorded 15 minutes following intravenous injection of 1 or 2 grams of $CaCl_2$ to two groups of patients with presumably intact autonomic nervous systems were not signficantly different from one another, although control values were similar. Simultaneously, similar increases in Ca^{++}_P were measured following 1 or 2 grams $CaCl_2$. Possible explanations are the rapid deposition of Ca^{++}_P in body stores if perfusion of those areas is adequate, or rapid redistribution.

There appears to be a dual effect of intravenously administered $CaCl_2$ depending on the base line of autonomic tone. *Postoperative patients* respond to $CaCl_2$ with increase in mean arterial pressure resulting from an *increase in systemic vascular resistance* (fig. 30) while *patients under deliberate hypotensive anesthesia* (ganglionic blockade) respond with increase in mean arterial blood pressure resulting from *increase in stroke volume* (figs. 31-34). Precise definition of the factors that lead to this difference in responsiveness requires further investigation.

We have encountered extraordinarily low Ca^{++}_P in patients in "low-flow" states. This low-output state was evident from low systemic blood pressure or cardiac output, dependence of the circulation and urinary output on circulatory support, or, late during the course of critical illness, from ischemic changes of the intestinal mucosa (patients 3 and 6, table 9). Although the exact cause of disproportionally low Ca^{++}_P values remains obscure, several factors have been identified as possible causes. *In vitro* experiments (*cf.* p.54) have demonstrated that elevation of lactate, phosphate or citrate are associated with significant decrease of Ca^{++}. For example, at a lactate concentration of 4 mM/L this complexation was associated with a 20% decrease in Ca^{++} (fig. 20) while at a phosphate level of 3 mM/L an even larger decrease was observed (fig. 21). Elevated plasma citrate levels have been reported[15, 60, 61] to cause consistent decreases of Ca^{++}_P, resulting in significant hemodynamic changes. Our *in vitro* data (fig. 22) are in agreement. If indeed these metabolic changes are responsible for the decrease of Ca^{++}_P values in patients, they would be expected to occur in a state of metabolic acidosis. In only four of the patients in "low-flow" state, however, was a significant degree of metabolic acidosis present.

In vitro experiments have demonstrated that binding characteristics of commercial protein solutions are significantly different from those of native protein (fig. 23). Although we do not have data on acute changes in protein patterns in critically ill patients it is conceivable that this phenomenon may be a contributing factor to the low Ca^{++}_P values measured in these patients.

Our data on patients in "low-flow" state are consistent with a report on Ca^{++}_P changes that appeared following graded hemorrhage with shock in the animal model[6] suggesting that *a low Ca^{++}_P level is not necessarily contributory to the hemodynamic changes.* It is possible that in the presence of a "low-flow" state a decrease in Ca^{++}_P does not stimulate

parathormone release due to inappropriate parathyroid function. Animal experiments[33] have recently suggested that β receptors may be present in the parathyroid glands and that infusion of epinephrine or isoproterenol can stimulate parathormone release. This is consistent with our findings in patient 8 (table 9), in whom the observed effect may also be a result of increased blood flow secondary to isoproterenol administration.

Hypocalcemia, responding inappropriately to $CaCl_2$ administration has been described in the presence of hypomagnesemia[23, 29]. In our patients (*cf.* p.78), [Mg] ranged from 0.8 to 2.4 mEq/L. Failure of intracellular reception of the cyclic AMP message has recently been suggested[23] as a possible mechanism of hypocalcemia refractory to $CaCl_2$ administration. We cannot exclude this as an explanation for the intractable hypocalcemia observed.

Hyperglycemia may be observed in association with a number of conditions commonly encountered in critically ill patients including inadequate pancreatic perfusion, administration of epinephrine or hypocalcemia[76]. In patients 5 and 8 each of these factors alone or in combination may have been responsible for the elevated blood sugar levels observed.

It is of interest to note the absence of rise in P_I following $CaCl_2$ administration in all patients presented. This rise is usually observed by the endocrinologist following $CaCl_2$ administration and appears to be independent of kidney function.

Further study is required to clarify the clinical importance of abnormally low Ca^{++}_P observed in critically ill man and to validate the administration of high doses of $CaCl_2$ in an attempt to correct these Ca^{++}_P levels.

CHAPTER VI

SUMMARY

The relationship between plasma calcium concentration and hemodynamic function is poorly understood. Little is known about Ca^{++}_P patterns during and following anesthesia and operation. It has been generally assumed that in the absence of obvious alterations in endocrine function, acute changes in calcium homeostasis would occur in neonates, following infusion of citrated whole blood or in association with changes in hydrogen ion activity. Critical care experience has shown that significant, persistent and profound alterations in the maintenance of Ca^{++}_P can and do occur during periods of critical illness. Use of the calcium selective electrode has clarified the magnitude of these acute changes.

In Chapter I we have reviewed methodology for Ca^{++}_P determination and physiocochemical principles as they apply to potentiometry. The ionized fraction of plasma calcium is the physiologically active moiety as demonstrated originally by McLean and Hastings in frog heart experiments. Potentiometric Ca^{++} measurement was first suggested 45 years ago, but only recently has a practical method become available. Effects of other ionic species on Ca^{++} measurement have been discussed. The reproducibility of Ca^{++} measurement with the electrode was good. The span of the clinically encountered Ca^{++} concentrations is within the useful sensitivity range of the electrode.

In Chapter II the techniques for measuring Ca^{++}, [Ca], P_I, [Mg], T.P. concentrations and pH are described. Practical aspects of handling of samples without loss of CO_2 are discussed. The technique commonly used in the sampling of arterial blood for blood gas analysis proved to be practical for the collection and handling of blood samples in which Ca^{++}_P is to be measured. Ca^{++}_P values measured in venous blood samples obtained from 32 normal adult volunteers have been included and compared with values from the literature. Ca^{++}_P values in children (age range: 2-12 years) undergoing elective tonsillectomy or eye surgery were lower than those found in adult volunteers and lower than predicted (McLean-Hastings nomogram).

In Chapter III the *in vitro* and *in vivo* effect of heparinization and of coagulation were described. In contrast to data from the literature, heparin does not alter Ca^{++}_{P} provided no volume change occurs secondary to the addition of heparin solution. The *in vivo* effect of heparin was evaluated by measurement of Ca^{++}_{P} prior to and following systemic heparinization of patients prior to cardiopulmonary bypass in preparation for open heart surgery. No significant difference was found. Ca^{++}_{P} was identical with Ca^{++}_{WB} ; practical aspects of measurement in whole blood limit its application at present.

In Chapter IV the effects of hemolysis, magnesium, pH, different anions and temperature changes on Ca^{++}_{P} measurement are discussed and the possibility of an arterio-venous difference considered. The effect of hemolysis was investigated by addition of increasing amounts of hemolysates to plasma samples of known Ca^{++}_{P} concentration. Hemoglobin levels up to 10 g/100 ml do not significantly alter Ca^{++}_{P} . The effect of magnesium as a possible source of interference with Ca^{++}_{P} measurement was determined by adding increasing amounts of crystalline magnesium chloride to plasma samples of known Ca^{++}_{P} . It was found that a [Mg] level of up to 9 mEq/L does not significantly interfere with Ca^{++}_{P} measurement. Above that [Mg] level, depression of apparent Ca^{++}_{P} occurs. An appreciable effect of pH is shown, illustrated by titration curves for both normal adult volunteers (*in vitro* titration curves) and patients with acquired heart valvular disease (*in vivo* titration curves). The slopes of these titration curves were similar. The effect of temperature on Ca^{++}_{P} measurement was investigated by maintaining the electrode at 25 and 37°C. A good agreement was found between values measured at these temperatures. This may be explained by the opposing effects of pH and temperature *per se* on the equilibria between the physicochemical forms of calcium. Although a systemic difference in Ca^{++}_{P} was not expected, we have investigated this possibility, for appropriate interpretation of Ca^{++}_{P} values measured in arterial and venous blood samples. Correcting for the difference in pH, the a.-v. difference in Ca^{++}_{P} was not statistically significant. Recognition of inappropriately low Ca^{++}_{P} values encountered in critically ill patients prompted investigation of complexation of calcium by various anions. Lactate and phosphate may decrease Ca^{++} by ion-pair formation while citrate is a chelating agent. The titration curves of these anions with Ca^{++} suggest that significant complexation can occur in

clinical conditions such as metabolic acidosis (lactate), renal failure (phosphate) or rapid infusion of whole blood (citrate). At a lactate concentration of 4 mM/L, approximately 20% of the calcium present was complexed; at a phosphate level of 3 mM/L there was even more complexation. Significant chelation by citrate was observed throughout the range of citrate concentrations studied. The clinical significance of these findings remains to be defined and requires measurement of plasma levels of these anions and of Ca^{++}_{P} simultaneously.

The effect of proteins was evaluated by addition of $CaCl_2$ to protein solutions prepared from blood drawn from a normal adult volunteer and to commercial plasma protein fractions. The commercial proteins displayed a significantly higher affinity for calcium ions than native protein.

In Chapter V critical care experience with Ca^{++}_{P} measurement is described. Ca^{++}_{P} values measured prior to and following induction of anesthesia in patients undergoing elective abdominal surgery were not significantly different from one another, but lower than those found in adult volunteers, as were Ca^{++}_{P} values measured following admission to recovery room. Although a positive inotropic action of the calcium ion has been assumed, there are no *in vivo* data available to demonstrate its mechanism of action in man. Postoperative patients respond to $CaCl_2$ injection (200 or 400 mg per minute, total dose 1 or 2 grams) by an increase in *systemic vascular resistance* resulting in an increase in mean arterial blood pressure, while patients under deliberate hypotensive anesthesia respond to $CaCl_2$ administration by an increase in *stroke volume*. The response of postoperative patients to i.v. injection of 1 gram $CaCl_2$ was similar to that following injection of 2 grams, both in terms of elevation of Ca^{++}_{P} and in terms of elevation of arterial blood pressure. The minimum effective rate of $CaCl_2$ administration was observed to be 100 mg per minute. The lowest Ca^{++}_{P} values encountered in this laboratory were measured in patients in "low-flow" states. This severe and intractable hypocalcemia, not necessarily associated with transfusion of large amounts of citrated whole blood, was apparent in association with borderline hemodynamic function. These findings were made in the course of routine monitoring of Ca^{++}_{P} levels in critically ill patients. Characteristics of the "low-flow" state included inappropriately low Ca^{++}_{P} values and inadequate responsiveness to intravenously administered $CaCl_2$. Definition of a "normal" response to $CaCl_2$ administration must await further studies in adult volunteers.

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APPENDIX

WORKING PROCEDURE FOR IONIZED CALCIUM MEASUREMENT

In the present study we have used the Orion (model 99-20) calcium selective flow-through electrode, connected to a flow-through reference electrode and a constant infusion pump (model 88-20). The calcium selective electrode was assembled according to the instructions supplied.[100] Potentials were read from a voltmeter (model 801).

To establish the relationship between electrode potential, E (mV) and calcium activity, $A_{Ca^{++}}$ (mM/L), aqueous standard solutions with concentrations of 0.25, 0.50, 1.0 and 2.0 m$\underline{M}$ $CaCl_2$ were used to construct a calibration curve.[100] These solutions also contained 150 mM/L NaCl and their pH fluctuated around 5.8.

The electrode was stored dry. Preliminary conditioning consisted of alternating the 1 m$\underline{M}$ $CaCl_2$ standard solution with a random plasma sample until consecutive readings of the standard solution agreed within 0.1 mV. The standards were then run through the electrode system and this procedure was repeated until each potential reading was stable within 0.1 mV. The minimum acceptable potential difference between consecutive standards was 5.5 mV; the electrode was then disassembled, cleaned with petroleum ether and reassembled. Greater electrode stability was noted after a number of plasma samples had been measured and with the intermittent use of a 2 m$\underline{M}$ $CaCl_2$ solution containing trypsin and triethanolamine (*cf.* p. 22). The 1 m$\underline{M}$ $CaCl_2$ standard was run between each passage of plasma and the electrometer reading rechecked to correct for electrode drift. The time required for calibration was approximately 30 minutes, readings of each sample required an average of 7 minutes.

Plasma samples were prepared from whole blood collected in plastic syringes the dead spaces of which were prefilled with a sodium heparin solution*(*cf.* p. 23). The pH of the whole blood sample was measured and the tip of the syringe covered with a stainless steel cap. The syringe was placed in a centrifuge with the tip up and the blood was centrifuged at 1500 *g* for 15 minutes. The plasma was transferred into another plastic syringe through a plastic 3-way stopcock. This eliminated changes of pH secondary to loss of CO_2. A tuberculin syringe was filled from the plasma-containing syringe through the 3-way stopcock

* "Liquaemin" (1000 I.U. per ml) Organon, West Orange, N.J.

and placed in the pump. After the first electrometer reading was made, the 1 m$\underline{M}$ $CaCl_2$ standard solution was passed through the electrode system, permitting recognition and correction of electrode drift. The plasma sample was then again passed through the electrode, followed by the 1 m$\underline{M}$ $CaCl_2$ standard solution. This procedure was repeated until the readings of the plasma sample agreed within 0.1 mV. Ca^{++}_P was found by interpolation of the mV reading on the calibration curve.

At the end of the day, the trypsin-triethanolamine containing $CaCl_2$ solution was passed through the electrode system, followed by passage of air.

CURRICULUM VITAE

Lambertus Johannes Drop was born in Vlaardingen on 10 April 1942 He received undergraduate education at *Chr Lyceum,* Zeist (1953-1955) and at *Lorentz Lyceum* Eindhoven (1955-1959), graduating in June, 1959 In September, 1959 he was enrolled in medical school at *Rijksuniversiteit,* Leiden He did a clinical clerkship in urology at *Landeskrankenhaus,* Klagenfurt a W , Austria (1962) and in general surgery, at *Kantons spital,* Glarus, Switzerland (1963) Following graduation in September, 1964 *(doctoraal examen),* he took rotating internships at the teaching hospitals of the medical school *(Stichting Klinisch Hoger Onderwijs)* in Rotterdam In November, 1966 he was awarded the licensure diploma *(arts examen)* From December, 1966 until January, 1968 he was an *interne* in the *service de médecine de l'Hôpital d'Yverdon,* Switzerland and from January, 1968 until December, 1968 a resident in surgery at West Virginia University Medical Center in Morgantown, West Virginia In January, 1969 he started a 3-year training program in anesthesia at Massachusetts General Hospital, Boston, Massachusetts during which time the present study was initiated From January, 1972 he was a member of the anesthesia staff at the Massachusetts Eye and Ear Infirmary, Boston, Massachusetts In October, 1973 he was certified as a Diplomate of the American Board of Anesthesiology He is presently in the Department of Anesthesia at Massachusetts General Hospital, Boston, Massachusetts

STELLINGEN

I

Since marked fluctuations in plasma ionized calcium levels can and do occur in critically ill patients, it is important to monitor these levels closely in such patients.

II

The lowest plasma ionized calcium level at which myocardial contraction ceases differs between species.

III

It is desirable that a state of deliberate hypotensive anesthesia be reversed prior to wound closure. Calcium chloride should be considered for this purpose since this drug restores arterial blood pressure by increase of flow.

IV

The hemodynamic effect of calcium chloride in man can be explained by different mechanisms of action.

V

Significant changes in the affinity of hemoglobin for oxygen are observed in the presence of variously substituted benzoic acids, as demonstrated by a shift of the oxyhemoglobin dissociation curve to the right.

J. Invest. Rad. 8:191, 1973

VI

In cases where a physician's clinical impression is contradicted by repeated objective laboratory measurements, a new impression should be formed according to appropriate interpretation of those measurements.

VII

Regional perfusion with anti-metabolites is indicated in the treatment of inoperable malignant melanoma.

Ann. Surg. 176:900, 1972

VIII

In patients with acute respiratory failure, significant improvement in arterial oxygenation can be achieved by application of controlled ventilation with positive end-expiratory airway pressure. This mode of therapy is not necessarily associated with significant changes in hemodynamic function.

J. Clin. Invest. 52:2315, 1972

IX

As the noxious effect of high environmental noise levels on various human organ systems is becoming increasingly appreciated, preventive measures are urgently needed.

Report to the President
on Noise. Environmental
Protection Agency
Washington, D.C. 1971

X

The Bach cantata may be counted among the highest and purest musical expressions of Christian faith.

Alfred Dürr: Die Kantaten von
Johann Sebastian Bach
Bärenreiter Verlag, 1971

XI

Statements ("stellingen") traditionally accompanying a doctoral thesis mainly serve a folkloristic purpose and do not necessarily indicate or prove a wide scope of scientific interests on the part of the doctoral student ("promovendus").

Interdependence between plasma ionized calcium
and hemodynamic performance. 30-V-1974